

From science to regulatory impact.

*A perspective from Horizon2020 projects
EU-ToxRisk and RISK-HUNT3R.*

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EU-ToxRisk: Interdisciplinary collaboration between academia, industry and regulators.

EU-ToxRisk Goal: evaluate if and how NAMs can support chemical safety assessment.

[:::] EUTOXRISK

- ~30 MEuro
- 38 European partners + 1 US partner
 - Academia & Research Institutes
 - Small & Medium-Sized Enterprises (SMEs)
 - Industry (chemical, pharmaceutical, cosmetic)
 - Regulators & other Stakeholders
- Scientific Advisory Boards: NTP, EPA, ECHA, Bayer
- Regulatory Advisory Board: ECHA, EFSA, SCCS, EMA, OECD



How to apply NAM for hazard and risk assessment?

Case studies as drivers

A series of case studies based on co-creation with focus:
how to apply NAM in a read across assessment context

Case study reports evaluated by **industry and regulatory stakeholders** for consultation and advice:

- ☐ RAB (Regulatory Advisory Board) EU-ToxRisk (Jan 2019)
- ☐ Expert Workshop Espoo with key stakeholders (May 2019)
- ☐ OECD IATA Case Study Project (April 2019)



Impact of systematic stakeholder interactions: advisory document on regulatory requirements for acceptance of NAM-assisted RAx

Recommendations of the EU-ToxRisk Regulatory Advisory Board (RAB) on how to document case studies for regulatory evaluation

27 April 2018

Archives of Toxicology (2019) 93:3643–3667
<https://doi.org/10.1007/s00204-019-02591-7>

GUEST EDITORIAL



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Towards grouping concepts based on new approach methodologies in chemical hazard assessment: the read-across approach of the EU-ToxRisk project

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Meeting Report

NAM-Supported Read-Across: From Case Studies to Regulatory Guidance in Safety Assessment

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The use of new data is a main goal of the collaboration with the European Committee on Toxicology (ECT) in the USA, as well as in other countries. The discussion was held to consider the use of NAMs as well as chemical dynamics, NAMs of similarity or of relative data correct the uncertainty. An interesting way of avoiding the data gap in every case, regardless of the case is still an open question.

14 Workshop Report*

Internationalization of Read-Across as a Validated New Approach Method (NAM) for Regulatory Toxicology

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Prediction of a 90 day repeated dose toxicity study (OECD 408) for 2-Ethylbutyric acid using a read-across approach to other branched carboxylic acids.

Read-across based filling of developmental and reproductive toxicity data gap for methyl hexanoic acid.

Identification and characterization of parkinsonian hazard liability of deguelin by an AOP-based testing and read across approach

Waiving of repeat-dose neurotoxicity study (TG 424) for azoxystrobin based on Read-Across to other strobilurins

1 Abstract / Synopsis / Executive summary

This section should provide a brief overview of the case study, including the objectives, concepts, methodologies, outcomes, and conclusion in about 300 words.

The synthetic strobilurin fungicides are derived from the naturally occurring strobilurin A and B. The strobilurins bind to the quinol oxidation site of cytochrome b of complex III of the mitochondria which is also their fungicidal mode of action. There are some signals of potential neurotoxicity from in vitro studies by a CILI-mediated mechanism.

The objective of this read-across case is to justify the waiving of an OECD TG 424 study for azoxystrobin by means of NAM data. The source compounds are other strobilurin fungicides. The formation of the category is based on the hypothesis that the compounds share similar chemical structure, similar pesticidal mode of action, similar toxicophore, similar neurotoxic potential and similar toxicokinetics to azoxystrobin. The source compounds chosen were pyraclostrobin, picoxystrobin, trifloxystrobin, and kresoxim-methyl. Furthermore, testing was conducted on Antimycin A, a well-established CILI inhibitor with neurotoxic effects, which serves as a reference compound for this mode of action. The degree of in vivo inhibition of the mitochondrial respiratory system depends on the respiratory activity and thus the tissues like brain can be more susceptible.

Existing regulatory in vivo data was collected for the source and target compounds with a focus on ADME, neurotoxicity as well as target organ toxicity data. The source compounds do neither show signs of neurotoxicity in neurotoxicity studies nor in other repeat dose toxicity studies.

The scientific hypothesis is: Can the absence of a neurotoxic potential (as detected with a TG424 study) mediated by inhibition of Complex III of the mitochondria be predicted by toxicodynamic and toxicokinetic NAM data?

The hypothesis is supported by mechanistic data, anchored to a putative AOP (based on the recently OECD adopted AOP on CILI inhibition leading to parkinsonian disorder), and kinetic PBTK data. Thus, the following data was obtained: physicochemical similarity (toxicokinetic), effects on oxygen consumption (mitochondrial complexes and whole cells), effects on mitochondrial membrane potential, cellular damage measured by effects on glycolysis and cell viability in three different cell types including neuronal cells, neuronal degeneration and neurite outgrowth.

The overall structural similarity of the compounds, although having the same pesticidal mode of action and toxicophore is less.

Inhibition of CILI complexes measured by oxygen consumption, by the target compound azoxystrobin seemed to be slightly less strong than by the source compounds pyraclostrobin and picoxystrobin, while antimycin A resulted in a much stronger inhibition. This was confirmed with whole cells as well. Effects on membrane potential were marked by Antimycin A and orders of magnitude less with the target and source compounds. Effects on glycolysis and cell viability were similar between the compounds. The target compound was negative in the neurite outgrowth assay in SH-SY5Y cells, while some of the source compounds did show weak effects, and neither the target nor the source compounds were regarded as neurotoxic in the neurite out assay in LHMES cells.

Enrico Montali, Frederic Bois, Paul Jennings, Rabaa Ghawel, Lilian Garcia, Thomas Ermer, ...

2-Ethylbutyric acid (2-EBA) has to be tested in more than 100 in vivo studies. The use of a read-across approach to other branched carboxylic acids is a consistent trend identified in the in vivo studies in silico models are used in the characterization.

a short chain, branched carboxylic acid with different structural analogues that have this same source chemicals to MHA. Selected: 2-ethylhexanoic acid (PHA), 2-methylpentanoic acid (4-pentenoic acid (4-ene-VPA)), proved to be clear reproductive toxicity as not being toxic to the neural tube defects upon in conclusion on the reproductive toxicity selected source chemicals in fish Embryo Test (ZET), mouse test model (UKN1), and a series of models to calculate effective dose. With these new approach, it correctly predict the in vivo toxicity could be used to predict the in vivo toxicity to further explore the series of aliphatic carboxylic acids. The absence of in vivo data, use activity in ZET, mEST, and stating target leading to neural toxicity.

model was established, based on the data which guided the use of PBPK models were used to predict in vitro clearance (CL_{int}, _{int}). The model predicted good predictive results. Based on this proof of concept for all analogues.

describing the development of the model were compiled from the network. The AOP network

endpoints covered, as

range to the class of insecticides, and pesticide. The two cases. Since 2008



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Taking the learnings into a new program: RISK-HUNT3R.



*“**RISK** assessment of chemicals integrating **HU**man-centric
Next-generation **T**esting strategies promoting the **3Rs**”*

- €23M, H2020 project (2021 –2026) – start 1 June 2021
- 37 EU partners (coordinator: Leiden University)
 - Academia & research institutes
 - **Regulatory → RIVM and BfR**
 - SMEs
 - **Large industry (BASF, Unilever, L’Oreal, Bayer CS, Inditex)**
- **Scientific and Regulatory Advisory Board (SRAB)**
- **Overall goal: Chemical risk assessment** based on **non-animal approaches** via **ab initio** safety evaluation, going **from hazard identification to full risk assessment**



ASPIS cluster – RISK-HUNT3R, ONTOX, PrecisionTox



ASPIS: “Animal-Free Safety Assessment of Chemicals: Project Cluster for Implementation of Novel Strategies”

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

- **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**



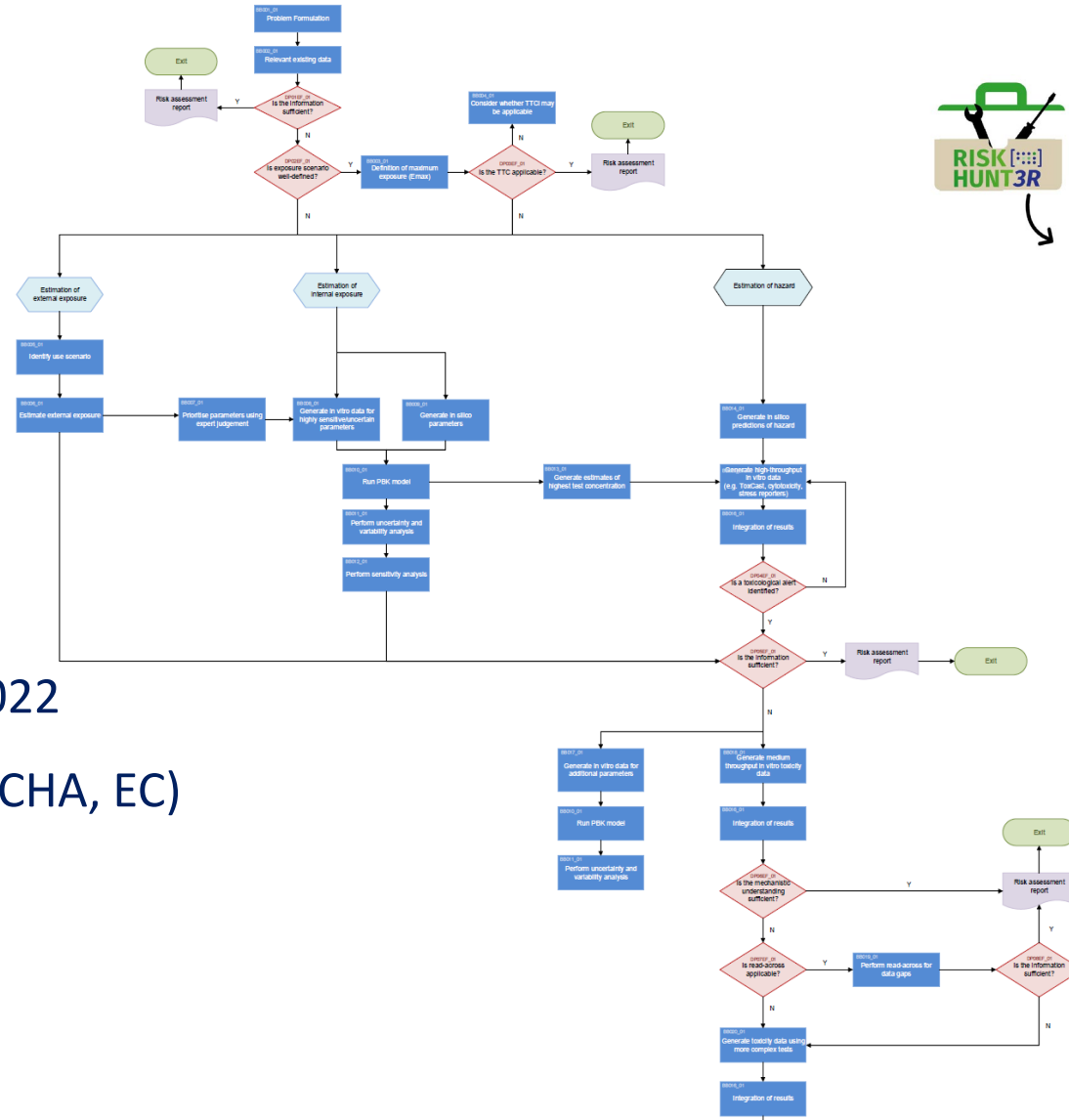
ASPIS NGRA framework: a roadmap to succes.....?

Roadmap for stakeholder engagement.

- ASPIS partner workshop 27 Oct 2022
- ASPIS Open Symposium 24-25 Nov 2022
- ASPIS Regulatory Forum (JRC, EFSA, ECHA, EC)
- Stakeholder workshop at JRC
- Case studies in ASPIS cluster



ASPIS NGRA workflow draft v1



Learnings, dilemmas and challenges for implementation of science-driven NAM-based NGRA.

- **Strong interactions** between scientists, SMEs, industry and regulatory agencies is essential.
- **Co-creation of regulatory relevant case studies** is essential to demonstrate NAM applicability for NGRA.
- **Mutual training of scientists, industry and regulators** critical to understand the opportunities, problems and requirements.
- ASPIS cluster projects, EURION cluster projects and PARC program places **high demand on stakeholder interactions**.
- Challenge is to **streamline the discussions** on NAM-based NGRA opportunities.
- **Free-up time of all stakeholders** will be essential to speed-up NGRA implementation in RA.

