FINAL REPORT

Project: Facilitate the regulatory acceptance and practical use of new approach methods in next-generation risk assessment

Vertrags-ID: 142005151 / 643-4/1/1

Including

Summary Technical Report D1[1]

Summary of the landscaping exercise assessing the status of integration and use of NAMs across sectorial frameworks

Summary Technical Report D2[1]

Summary of the gaps & needs analysis, barriers, opportunities, drivers for integration and use of NAMs across sectorial frameworks

Workshop Report D3[1]

Action plan for prioritization of regulatory-relevant scenario-based case studies

Technical Report D4[1]

Summary of the scenario-based case studies (initiation)

scaht
Swiss Centre for Applied Human Toxicology

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[1] according to Vertrags-ID: 142005151 / 643-4/1/1, chapter 7 “Termine”

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1 EXECUTIVE SUMMARY

The necessity and relevance of animal experiments for human health risk assessment are increasingly debated, particularly regarding whether they can be replaced entirely. While animal testing remains indispensable for evaluating systemic toxicity following repeated exposure (DFG, 2023), alternative approaches are rapidly advancing.

New Approach Methodologies (NAMs) already provide valuable mechanistic insights into modes of action, especially when integrated into conceptual frameworks such as Adverse Outcome Pathways (AOPs), Integrated Approaches to Testing and Assessment (IATA), and Defined Approaches (DA). These frameworks enable more scientifically robust risk assessments and help reduce animal use. Mechanistic understanding is already informing regulatory decisions, for example, in deriving threshold values or applying read-across methods when substance-specific data are limited.

Despite these advantages, the regulatory uptake of NAMs still lags behind their technical potential, constrained by legal, motivational, and economic factors. This report demonstrates that NAMs have the potential to transform chemical risk assessment, provided there is consensus on their “fit-for-purpose” use. The “fit-for-purpose” principle acknowledges that validation requirements must align with the intended regulatory context, emphasizing flexibility and proportionality in the validation process.

Insights from literature, expert consultations, and NAM case studies across chemical sectors and regulatory settings reveal strong and growing interest in human-relevant NAMs and rapid progress in their development. However, their implementation depends on the adaptability of existing regulatory frameworks and on broader cultural change at individual, institutional, and societal levels. To fully leverage NAMs, greater flexibility in testing requirements is needed, along with continuous education for regulators and stakeholders to clarify when and how specific NAMs can be applied and how their data should be interpreted.

Building scientific and regulatory confidence further requires clear guidance to streamline and accelerate validation processes, coupled with structured opportunities for early dialogue between method developers and regulatory agencies to ensure that data needs are met.

Although successful NAM-based submissions exist, regulatory acceptance remains uneven—frameworks under the US EPA, for instance, appear more accommodating than those implemented by ECHA. Achieving global acceptance will require sustained international collaboration and cross-sector coordination to harmonize standards and advance the best science efficiently.

The Swiss Federal Office for Public Health (FOPH) largely follows European law when it comes to chemical regulations. It also supports Swiss research, for example to strengthening expertise in regulatory toxicology, including NAMs and AOPs, within both the academic and regulatory environments. The Swiss Centre for Applied Human Toxicology (SCAHT) acts as an interface of research, regulation, and education. Supported by FOPH and to fulfil its role of a scientific interface, the SCAHT organized five webinars and an in-person workshop aiming at i) educating Swiss regulatory agencies and academia on next generation risk assessment (NGRA), AOPs and IATAs and ii) developing ideas for Swiss case studies with problem formulations to be tackled with NAMs.

Two case studies are presented to illustrate future directions in applying NAMs: 1. Health consequences of bisphenol TMC (BPTMC) exposure and 2. Metal mixture exposure and the consequences for brain development. These case studies were deliberately selected to represent contrasting scenarios and demonstrate the broad applicability of NAM-based approaches. The BPTMC case study reflects a data-poor situation in which literature review, AOP mapping, and the potential to apply read-across were explored. In contrast, the developmental neurotoxicity of heavy metals is well established; thus, the core challenge in this case study was to determine how an NGRA-like workflow could be adapted to evaluate mixture risks using NAMs.

For each case study, the report examines a workflow tailored to its specific needs and identifies gaps that must be addressed for future data integration. Both case studies require additional development and more robust datasets before regulatory conclusions can be drawn. These efforts will continue in upcoming initiatives, including the PARC project.

2 ABBREVIATIONS

Table 1: Abbreviations

Abbreviation	Term
3R	Replacement, Reduction, Refinement
3RCC	Swiss 3R-Competence Center
AOPs	Adverse Outcome Pathways
ASPA	Alternative Safety Profiling Algorithm
DA	Defined Approaches
DART	Developmental and Reproductive Toxicity
DIP	Data Interpretation Procedure
DNT	Developmental Neurotoxicity
EAWAG	Swiss Federal Institute of Aquatic Science and Technology
EC	European Commission
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EU	European Union
EURL ECVAM	European Union Reference Laboratory on Alternatives to Animal Testing
FOPH	Federal Office of Public Health
FSVO	Federal Food Safety Veterinary Office
GD	Guidance Document
HBM	Human Biomonitoring
HTS	High-Throughput Screening
IATA	Integrated Approaches to Testing and Assessment
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ISTNET	International Stakeholder Network
IVB	*In Vitro* Test Battery
IVIVE	In Vitro to In Vivo Extrapolation
JRC	Joint Research Centre
KC	Key Characteristics
KEs	Key Events
KERs	Key Events Relationships
MIEs	Molecular Initiating Events
MoA	Mode of Action
NAMs	New Approach Methodologies
NFA	Network Formation Assay
NICEATM	Interagency Center for the Evaluation of Alternative Toxicological Methods
NGRA	Next Generation Risk Assessment
OECD	Organisation for Economic Co-Operation and Development

OECD ESCA	Advisory Group on Emerging Science in Chemicals Assessment
PARC	European Partnership for the Assessment of Risks from Chemicals
PBK	Physiology-Based Kinetic
PoD	Point of Departure
QSAR	Quantitative Structure-Activity Relationship
REACH	Regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals
SCAHT	Swiss Centre for Applied Human Toxicology
UN GHS	Globally Harmonized System of the United Nations
US FDA	United States Food and Drug Administration
US EPA	United States Environmental Protection Agency
WHO	World Health Organization
WoE	Weight-of-Evidence

3 SCOPE OF THE PROJECT

This report summarizes the results of the SCAHT activities conducted during the three years of the project. It summarizes two previous reports (D1 and D2) on activities on the current status of NAMs implementation in regulatory human health risk assessment, webinar/workshop activities on NGRA, AOPs and NAMs (D3) and development of Swiss case studies to be elaborated in the future (D4).

The findings are compiled together in four parts of this final report:

- **Summary of technical Report D1**: A summary of the landscaping exercise, which assessed the current integration and application of NAMs across various sectoral frameworks.
- **Summary of technical Report D2**: A summary of the gaps and needs analysis, identifying barriers, opportunities, and drivers for the integration and application of NAMs across sectoral frameworks.
- **Workshop Report D3**: Report on the webinar series and the workshop outcomes.
- **Technical Report D4**: Report on the initiation phase of two Swiss case studies.

The objectives of the first year were to describe the status quo of NAMs implementation in current risk assessment practices in Switzerland and to investigate the barriers and drivers of

regulatory acceptance and practical use of NAMs. This was achieved through a landscaping exercise, involving a literature search and expert consultation (Technical Report D1).

The objectives of the second year were to build on these insights and describe general and sector-specific barriers and expectations related to the use of NAMs, to use case studies to illustrate the current use of NAMs for regulatory purposes to gain a better understanding of the needs of risk assessors and regulators in different chemical sectors (Technical Report D2), and finally to develop an action plan for the SCAHT to further advance the implementation of NAMs for NGRA in the third year. A detailed list of the SCAHT activities agreed with the FOPH for the first, second and third project year can be found in Table 2.

Table 2: Tasks and measures for the first, second and third project year

Year #, Task #	Measures
Y1, T1	Assemble information in Switzerland and Europe on the current situation regarding the use of NAMs in specific regulatory frameworks through a literature review
Y1, T2	Conduct a qualitative expert elicitation with risk assessors in Switzerland on the acceptance and use of NAMs and the barriers and drivers thereof
Y1, T3	Conduct a quantitative expert elicitation with risk assessors in Switzerland on the acceptance and use of NAMs and the barriers and drivers thereof
Y2, T1	Identify key factors and drivers e.g., barriers, opportunities, benefits, incentives) that may hinder/facilitate the integration and use of NAMs in the regulatory risk assessment decision making process;
Y2, T2	Select specific examples from the outcome of the landscaping exercise for a more in-depth gaps & needs analysis, using real-life regulatory scenarios to identify gaps and needs at desk level;
Y2, T3	Identify commonalities & divergence in the use of NAMs across chemical sectors, using a generic, sector-agnostic approach as well as a sector-specific approach; further explore how these tools and methods could be translated from one field/discipline (e.g., human vs environment), chemical sector, or application context, to another;
Y2, T4	Develop an action plan for improving the regulatory uptake of NAMs in the risk assessment practice, which is a major goal at the end of the second year of the project (M24).

Year #, Task #	Measures
Y3, T1	In the third year of the project, the output of the gaps & needs analysis will be used in a workshop (MS3) to design two regulatory scenario-based case studies (pesticide/non-pesticide) with the aim of orientating future coordination and harmonization efforts across Swiss federal agencies for sharing best research-to-policy practice in relation to the use and regulatory uptake of NAMs.

4 METHODS

4.1 YEAR 1: LITERATURE SEARCH AND EXPERT CONSULTATION

In year 1, the study followed a human-centred mixed-methods approach combining a literature review with two sequential expert consultation activities. It built on ongoing European initiatives, including the JRC survey on NAMs under Regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and related PARC internal and external activities (see Textbox 1).

Textbox 1. The Swiss project in the European context

A parallel mixed-method approach was conducted at the European level. The results were published in three peer-reviewed papers and in PARC deliverables (Bearth et al. 2024a, b; Bearth et al. 2025a, b). These activities further informed the work done in the Collaboration to Harmonise the Assessment of Next Generation Evidence (CHANGE) (VKM, 2025) of the Norwegian Scientific Committee for Food and Environment (VKM). These activities offer a nuanced view of the role of NAMs in the global regulatory landscape. The adoption of NAMs in regulatory toxicology is limited not necessarily by the science itself, but by system-level barriers. Key inhibitors include infrastructure and processes designed for animal data, misaligned incentive structures, and lack of trust and collaboration among stakeholders. Promoters include appropriate knowledge and skill exchange formats and collaboration, supportive infrastructure and processes, and flexible regulatory processes and an institutional culture that accepts innovative evidence, if scientifically appropriate. Systemic change, not just more new methods, is needed for NAMs to deliver real regulatory and public-health benefits.

The literature review covered scientific, grey, and project reports to identify definitions, current research, and knowledge gaps. Based on these findings, a semi-structured interview guide was developed for 30-minute expert interviews with Swiss risk assessors and regulators from federal offices, academia, and industry. Thirteen experts (N = 13) were recruited through theoretical sampling to ensure diversity in expertise. Interviews were conducted online (30–70 min), not recorded to ensure openness, and documented through written notes for thematic analysis.

An online survey subsequently validated and extended the qualitative insights. It targeted Swiss risk assessors from regulatory, industrial, academic, and consultancy settings, recruited through SCAHT networks and snowball sampling. The questionnaire, approved by the ETH Zurich ethics board (EK-2023-N12), covered socio-demographics, familiarity and use of NAMs, perceived barriers and drivers, and individual preferences. It included open and closed questions and separate branches for human health and environmental assessors.

The survey ran from April to July 2023 (median duration = 27 min) and was completed by N = 51 participants from Switzerland (37 human health, 14 environmental; 22 male, 19 female, 3 no answer; M_{age} = 51, SD = 12). Most respondents worked in regulatory agencies (n = 22), followed by industry (15), academia (7), and consultancy (7). Professional experience ranged from 3 to 45 years, and participants indicated the EU legal frameworks most relevant to their work (see Table 3).

Table 3: Participants' area of expertise and legal frameworks (multiple responses possible, *N* = 51).

	Human health risk assessor	Environmental risk assessor	Total
Plant Protection Products Regulation (EC) No 1107/2009	9 (24%)	8 (57%)	17 (33%)
Biocidal Products Regulation (EC) No 528/2012	8 (22%)	5 (36%)	13 (25%)
REACH Regulation (EC) No 1907/2006	16 (43%)	4 (29%)	20 (39%)

	Human health risk assessor	Environmental risk assessor	Total
Classification, Labelling and Packaging of substances and mixtures (CLP Regulation (EC) No 1272/2008	11 (30%)	4 (29%)	15 (29%)
Medicinal products for human use Directive 2001/83/EC	12 (32%)	0 (0%)	12 (24%)
Veterinary medicinal products Regulation (EU) No 2019/6	3 (8%)	0 (0%)	3 (6%)
Feed Additives Regulation (Regulation (EC) No 1831/2003	6 (16%)	1 (7%)	7 (14%)
Cosmetic Products Regulation (EC) No 1223/2009*	6 (16%)	-	-
Medical Devices Regulation (EU) No 2017/745*	4 (11%)	-	-
Food Additives Regulation (EC) No 1333/2008*	11 (30%)	-	-
Food Contact Materials Regulation (EC) No 1935/2004*	6 (16%)	-	-
Novel Foods Regulation (EU) No 2015/2283*	6 (16%)	-	-
Construction Products Regulation (EC) No 305/2011*	0 (0%)	-	-
Water Framework Directive+	-	5 (36%)	-
Integrated Pollution Prevention and Control Directive+	-	0 (0%)	-
Marine Strategy Framework Directive (MSFD) (2008/56/EC)+	-	0 (0%)	-
The Convention for the Protection of the Environment of the North-East	-	0 (0%)	-

	Human health risk assessor	Environmental risk assessor	Total
Atlantic (Oslo and Paris Convention; OSPAR Convention)+			
Other (e.g., Swiss Chemicals Act (SR 813.1), Swiss Chemicals Ordinance (SR 813.11))	7 (19%)	3 (21%)	10 (20%)
Total	37 (100%)	14 (100%)	51 (100%)

*Note. *: only shown to human health risk assessors; +: only shown to environmental risk assessors.*

4.2 YEAR 2: CASE STUDIES

Barriers to the implementation of NAMs in regulatory toxicology were identified through a review of scientific publications and reports from international expert meetings. Through its active involvement in expert groups working on NAM integration into regulation, SCAHT was able to validate the findings from the literature and discuss possible approaches and solutions with international partners. SCAHT used both, a generic, sector-agnostic approach as well as a sector-specific approach (e.g., bulk chemicals, plant protection products, cosmetics) to conduct a gaps & needs analysis. SCAHT identified case studies on the use of NAMs to meet regulatory requirements for the assessment of chemicals in the different sectors to highlight the key factors and major drivers that hamper and may facilitate a successful integration/uptake of NAMs in the regulatory risk assessment practice.

The result of the gap and needs analysis and the insights from the case studies is an action plan showing what the SCAHT will do to further advance the implementation of NAMs in regulation.

4.3 YEAR 3: WEBINARS, WORKSHOP AND CASE STUDY SELECTION

The goal of year 3 of the contract was i) educating on NGRA, AOP and IATA and ii) identifying Swiss case studies for further elaboration. Therefore, the SCAHT utilized its contacts for gaining experts in the fields. Five speakers, four external ones and one SCAHT internal speaker designed the five webinars and the in-person workshop (Table 4).

Table 4: Speakers designing the educational seminars and the in-person workshop on NAM education, training and Swiss case study identification.

	Affiliation	Topic
Predrag Kucic, PhD	Unilever	NGRA
Andrea Terron, PhD, VMD	EFSA	AOP
Iris Mangas, PhD	EFSA	IATA
Jean-Lou Dorne, PhD	EFSA	PBK modelling
Ellen Fritsche, MD	SCAHT	AOP

The webinars were designed as lunch seminars with 30-40 min talks and the opportunities to ask questions. The in-person workshop spanned a whole day with short refreshing talks of the respective speakers (Table 4) in the morning and breakout groups for case study discussions in the afternoon. The day was concluded by a common discussion on case study prioritization and followed up by case study definition.

4.4 YEAR 3: CASE STUDIES SET-UP

To tackle the case studies, the AOP as a primary tool in regulatory risk assessment using NAMs was utilized. Therefore, putative key events (KE) were identified through a non-systematic literature review and database check. For the literature review, PubMed and Web of Science was the primary data base. For the data bases we identified ToxCast, CompTox Chemicals Dashboard, the human biomonitoring dashboard, and AOP Wiki as relevant for the case studies.

5 TECHNICAL REPORT D1: SUMMARY OF THE LANDSCAPING EXERCISE ASSESSING THE STATUS OF INTEGRATION AND USE OF NAMS ACROSS SECTORIAL FRAMEWORKS

5.1 LITERATURE REVIEW

To date, no harmonized definition of NAMs exists in the European chemical regulatory landscape. Equally, an overview of how and to which extent they are applied for use in chemical risk assessment across the various regulatory sectors and application contexts is missing. The most relevant NAMs definitions have been summarized by the Joint Research Centre (JRC) and reviewed by the Organisation for Economic Co-operation and Development (OECD) Working Party on Hazard Assessment (WPHA) in (OECD, 2020). Several scientific or regulatory organizations worldwide have proposed their own working definitions of NAMs, among them the ECHA, the Nordic Council of Ministers, the USEPA, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), Health Canada, and the UK Committee on Toxicity of Chemicals. According to these different definitions, NAMs include grouping and read-across approaches, DA, in vitro test guidelines, and in silico models. According to some definitions of NAMs, animal tests are also included if they serve to reduce or refine another animal test (3R principle). Some definitions go beyond the use of NAMs for hazard assessment, by referring specifically to exposure or risk assessment. In silico approaches or models, computer models or computational methods are kept vague in these definitions (incl. quantitative structure-activity relationship (QSAR)). Commonly, the definitions recognise that a single approach or method cannot be used in isolation to reach sufficient reliability for regulatory acceptance, individual NAMs are complementary to each other to reach a decision given the specificity of the regulatory or application context, and that there is a need to go beyond the hazard dimension by integrating exposure as well.

For this project, the following working definition of NAMs was proposed:

New Approach Methodologies (NAMs) are defined as any valid and relevant technology, methodology, approach, strategies, or combination thereof that can provide information on chemical hazard, exposure, and risk assessment to reduce, refine or replace animal testing.

The literature review, which investigated the perspectives of risk assessors regarding NAMs and *in vivo* testing, revealed several articles of interest. The core insights of these articles are summarised below.

American mixed-method study (Zaunbrecher et al., 2017):

- Surveyed N = 1,381 risk assessors on the perceived viability and use of specific NAMs (mechanistic / high-throughput in vitro assays, small-animal in vivo assays, QSARs, biomarkers).
- Higher acceptance for NAMs in screening, prioritization, and comparative assessments than for establishing safety levels.
- Notable for distinguishing application contexts of NAMs rather than assessing general attitudes.

JRC/EURL-ECVAM/US EPA survey (Paini et al., 2017):

- Assessed international use of PBK models in science and regulatory submissions.
- Identified barriers: lack of standardized guidelines and diverse computing platforms.

Global AOP horizon-scanning survey (LaLone et al., 2017):

- Collected expert views on AOP development, best practices, and regulatory relevance.
- Emphasized the need for clear guidance, case-by-case evaluation of "fit-for-purpose," and positive case examples to support decision-making.

Social science perspectives (Mondou et al., 2020; Pain et al., 2020):

- Found that familiarity increases perceived viability of NAMs.
- Drivers: improved scientific understanding, new applications, cost reduction, efficiency gains.
- Barriers: insufficient validation, interpretive complexity, lack of standardization.
- Highlighted the field's overemphasis on innovation vs. adoption as a barrier to uptake.

Early foundational studies (Kraus et al., 1992; Slovic et al., 1995, 1997):

- Surveyed toxicologists' confidence in animal study validity.
- Findings: experts were split evenly on whether animal reactions reliably predict human responses.

Two core knowledge gaps were identified in the literature:

1. Most studies investigated the use and acceptance of NAMs overall, without differentiating the type of NAM or application context.
2. None of the studies focused on the psychological mechanisms that might drive or hinder the regulatory use and acceptance of NAMs. The expert consultation aimed at covering these two gaps and generating novel insights.

5.2 EXPERT ELICITATION

5.2.1 QUALITATIVE INTERVIEWS

A. Familiarity and definition of NAMs

As described in the methods section, 13 individuals from industry and regulatory authorities in Switzerland were interviewed concerning their familiarity with NAMs, their definition of NAMs, awareness and preferences of official NAM definitions, as well as their opinions on what is new/innovative, and whether in vivo test and exposure assessment should be included in the NAMs. The results of the interviews are summarized below:

Overall familiarity:

All interviewed risk assessors were familiar with the term "NAMs," though their definitions varied considerably depending on background, experience, and daily exposure to such methods.

Range of definitions:

- Simple definitions: NAMs as *alternative methods* to animal testing (in vitro, in silico, QSARs, mechanistic grouping, AOPs).

- Nuanced definitions: NAMs as *integrated approaches* (IATA, DA), guided by the 3R principle and including NGRA.
- Artificial Intelligence approaches: Rarely mentioned spontaneously; several agreed to include them under the NAM umbrella when prompted.

Awareness of official definitions:

Few were familiar with formal definitions (e.g., ECHA, OECD); most used personal "working definitions" shaped by professional practice.

Preference for broad definitions:

Many supported an *inclusive definition*, any 3R-aligned alternative to animal testing for hazard, exposure, or risk assessment, though some expressed reservations.

Key points of debate:

- **What counts as "new" or "innovative"**
  Some noted that in vitro alternatives have existed for decades and are routinely used within weight-of-evidence (WoE) frameworks.
- **Inclusion of in vivo methods**
  Some excluded refined or reduced animal tests, limiting NAMs to *replacement* methods only.
- **Inclusion of exposure methods**
  Opinions diverged: some restricted NAMs to hazard-based tools; others included exposure models (e.g., quantitative In Vitro to In Vivo Extrapolation (IVIVE), PBK) if aligned with 3R goals.

B. Status quo of the use of NAMs in risk assessment

According to the interviewees, while the degree of occurrence of NAMs in registrant dossiers and familiarity may vary per chemical sectors and regulatory application, overall use of NAMs in the daily regulatory human health risk assessment practice (Federal Office of Public Health FOPH, Federal Food Safety and Veterinary Office FSVO) remains limited, even more so in environmental risk assessment (Federal Office for the Environment FOEN, Agroscope, Ecotox

Centre). NAMs that are not validated and have not yet gained regulatory acceptance are not used for authorisation purposes.

NAM-based approaches exist for local toxicity (e.g., skin or eye irritation, skin corrosion), but no validated methods yet address systemic toxicity or allow Point of Departure (PoD) derivation based solely on NAMs. Recent breakthroughs show promise but remain limited in scope, such as:

- The US EPA ToxCast program (Dix et al., 2007) for predicting the potential for toxicity and prioritisation, utilising computational chemistry, high-throughput screening (HTS) and various toxicogenomic technologies.
- OECD accepted an in vitro oestrogen receptor binding assay for replacing the uterotrophic assay.
- US EPA has recently used omics data to derive a PoD.

NAMs are not yet used as primary evidence, but serve as supportive tools in a case-by-case, WoE approach. Their application depends on the regulatory context, data type, and purpose, with in vitro methods often combined with animal data. Guidance documents are essential for broader implementation, but readiness remains limited for omics, HTS, PBK modelling, IVIVE, and biomarkers, leaving animal and human data dominant in risk assessment.

A key discussion point was whether animal testing should remain the “gold standard.” Experts noted that uncertainties persist but may shift in nature, and that for some endpoints, such as neurotoxicity, comparative toxicity, or inhalation sensitizers, NAMs may already outperform animal models, illustrating that animals are not always reliable predictors of human biology.

C. Acceptance of NAMs in risk assessment

Discussions occasionally addressed the institutional roles influencing the adoption of NAMs. While most risk assessors were open to using NAMs, they emphasized that only OECD-validated methods and clear regulatory guidance could enable their integration into routine assessments. Acceptance also depends on alignment with EU-level rules and established approval processes.

Regulators supported the gradual transition away from animal testing but advocated a cautious, stepwise approach, combining NAMs with traditional data in WoE frameworks until NGRA matures. Ensuring that NAMs provide equivalent reliability and quality to animal data remains a key concern. Some experts noted that animal testing is still mandatory for certain sectors (e.g., pesticides, biocides) and doubted full replacement in the near term.

Industry representatives generally supported the “reduce and refine” principles but warned against overreliance on NAMs, citing stricter regulatory demands for clear-cut hazard classifications that reduce flexibility. They highlighted the challenge of maintaining comparable data quality and noted that recent regulatory shifts (e.g., OECD DA for Skin Sensitization under Classification, Labelling and Packaging (CLP)) impose new rigidity.

Finally, some experts questioned whether NAMs truly reduce animal use, noting that testing requirements have in practice increased. Others stressed better use of existing animal data (e.g., extended absorption, distribution, metabolism, and excretion studies) and warned that data gaps might undermine human and environmental protection despite good intentions.

D. Barriers and drivers of regulatory use and acceptance of NAMs

An overview and description of the barriers and drivers for regulatory uptake of NAMs mentioned by the interviewees is presented in Tables A.1 and A.2 in the Appendix A of the initial Technical report (D1 and D2, November 2024). Most barriers (Table A.1) are related and amplify each other (e.g., lack of incentive to incorporate and consider NAMs in regulatory risk assessment dossiers, resource restraints, and risk aversion).

To summarise, the barriers highlight systemic issues that cannot be changed by single risk assessors or single institutions but would require a system change. This system change needs to be accompanied by measures on the individual level, namely increasing trust, knowledge, and skills. Such a system change is currently ongoing spearheaded by the European Commission with the `European Roadmap towards phasing out animal experiments for chemical safety assessment` that was triggered by a European Citizens initiative (European Commission, 2023).

Textbox 2. Qualitative interviews with European risk assessors

In addition to the interviews conducted with Swiss risk assessors, the same interviews were conducted with European risk assessors (*N* = 11). Overall, it became clear that European and Swiss risk assessors are faced with similar challenges regarding the acceptance and use of NAMs at desk level. Differences in responses of the risk assessors can be traced more clearly to different legal frameworks that the risk assessor is operating under (e.g., plant protection products vs. REACH), their professional backgrounds and frequent tasks at desk level rather than their geographical location.

At international level, several organisations have taken the lead, and authorities from the United States of America Authorities (e.g., US EPA, US FDA) are transitioning towards NGRA to meet the political agenda phasing out animal testing by 2030. There is a push towards methods providing mechanistic information to support hazard-driven assessments, grouping and read-across, QSARs, and IATA-based approaches. The OECD is pushing the NAMs agenda for IATA and DA, QSARs, and more recently omics. In the EU, ECHA and EFSA have taken the lead on the regulatory front, in collaboration with numerous research initiatives and programmes (PARC, ASPIS, Eurion, Panoramix).

5.2.2 QUANTITATIVE SURVEY

A. Status quo of integration of classical methods and NAMs

Participants were introduced to the project's working definition of NAMs and asked to rate their familiarity and frequency of use across various methods. The results are depicted in Figures 1 and 2. In summary:

Familiarity of methods:

- Highest for in vivo assays (mammalian), DA, QSARs, and biomarkers (>75% somewhat or very familiar).
- Lowest for in chemico and omics methods (genomics, transcriptomics, metabolomics, proteomics) (>25% unfamiliar).

- Low familiarity was also reported for “other in vivo assays” (e.g., zebrafish embryo), likely due to unclear phrasing and restriction to human health assessors.

Frequency of use:

- In vivo assays were used most often (>60% frequently).
- Among NAMs, grouping/read-across, QSARs, PBK modelling, in vitro assays, and DA were most common (25–40% frequently).
- Omics methods were least used (38–50% never).

Additional suggestions:

Participants mentioned missing NAMs such as in vitro genotoxicity tests, PBK parameterization assays, bioaccumulation models, protein-ligand interaction models, and advanced human-derived in vitro systems. They also emphasized broader application contexts e.g., rapid screening, mixture toxicity, and WoE use beyond formal regulatory frameworks.

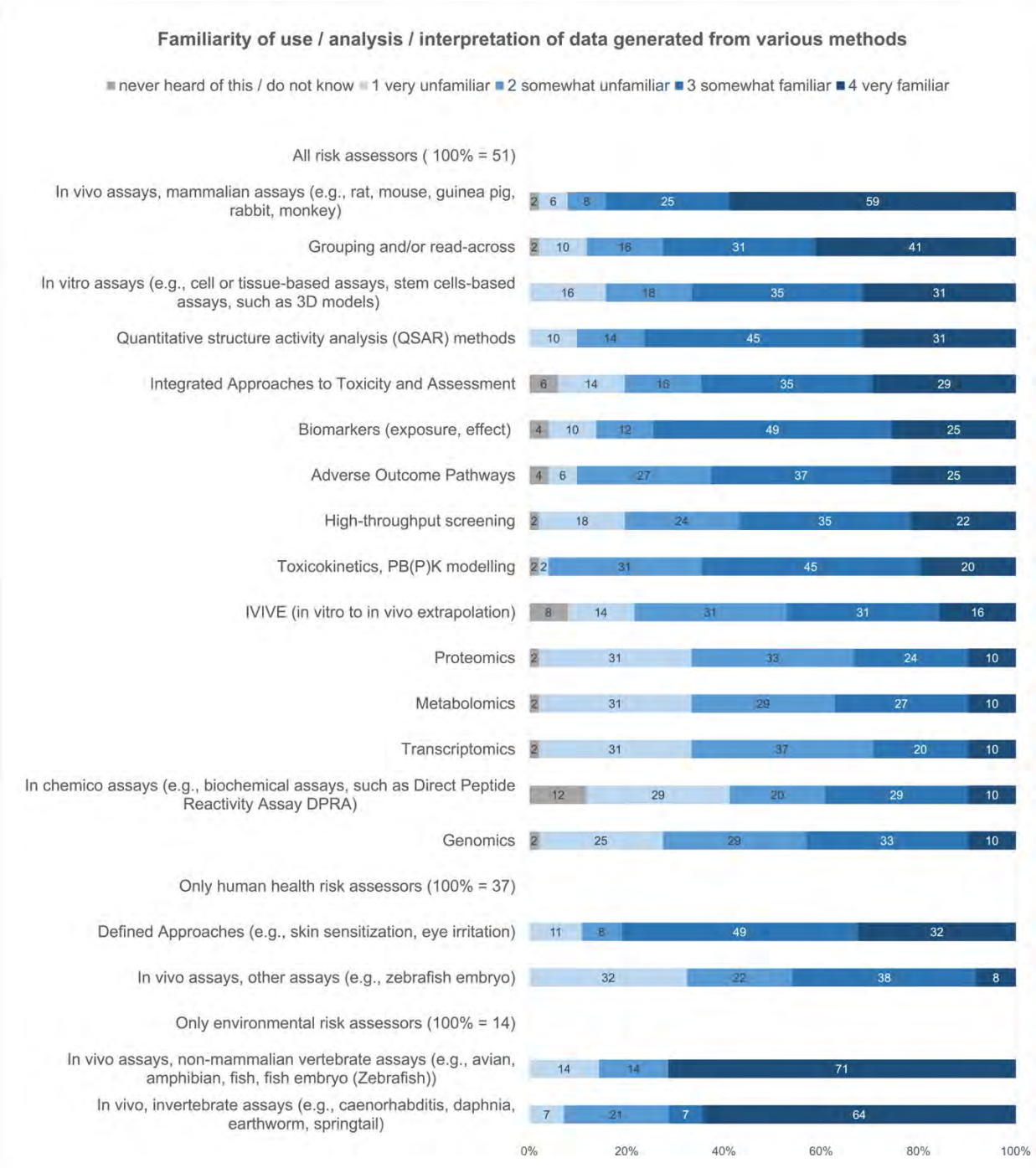


Figure 1. Familiarity of use / analysis / interpretation of data generated from various methods (examples for "high-throughput screening omitted in the Figure: in vitro cell-based assays, Oestrogen Receptor Model Assay (e.g., ToxCast, Tox21), in silico profiling assays using large chemical libraries (e.g., Pubchem, EPA CompTox)).

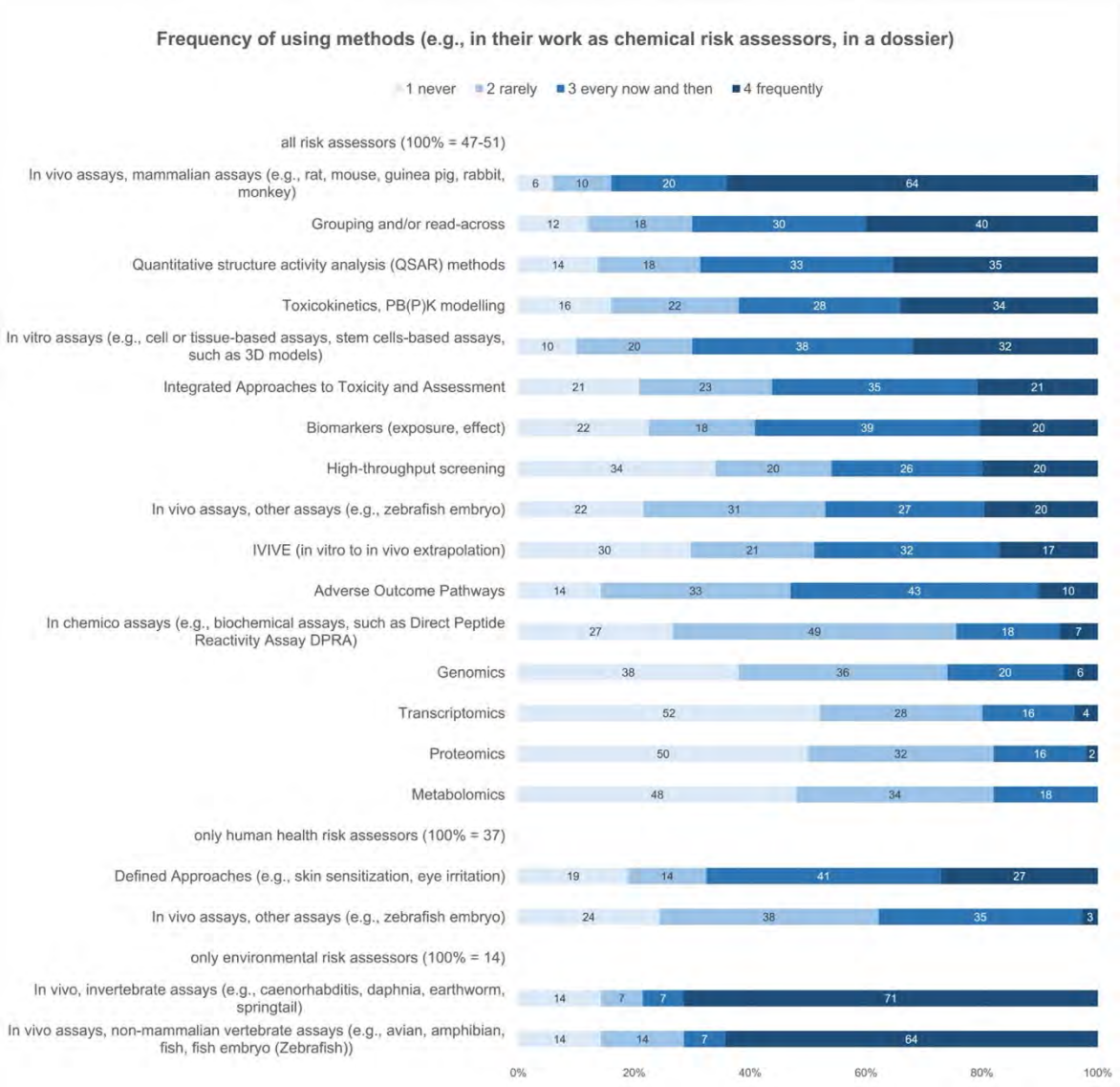


Figure 2. Frequency of using methods in their work as chemical risk assessors or in a dossier (examples for "high-throughput screening omitted in the Figure: in vitro cell-based assays, Oestrogen Receptor Model Assay (e.g., ToxCast, Tox21), in silico profiling assays using large chemical libraries (e.g., Pubchem, EPA CompTox)).

B. Barriers and drivers of the use and regulatory acceptance of NAMs

Participants were asked to rate the barriers and drivers of NAM use and regulatory acceptance, defined as the incorporation of validated NAMs into regulatory guidelines or, for non-validated NAMs, their case-by-case use in decision making. Using a 4-point relevance scale, most barriers were considered somewhat or very relevant by 74–91% of participants. Fewer (52–67%) viewed limited model accessibility, insufficient workforce capacity, and data management challenges as major barriers.

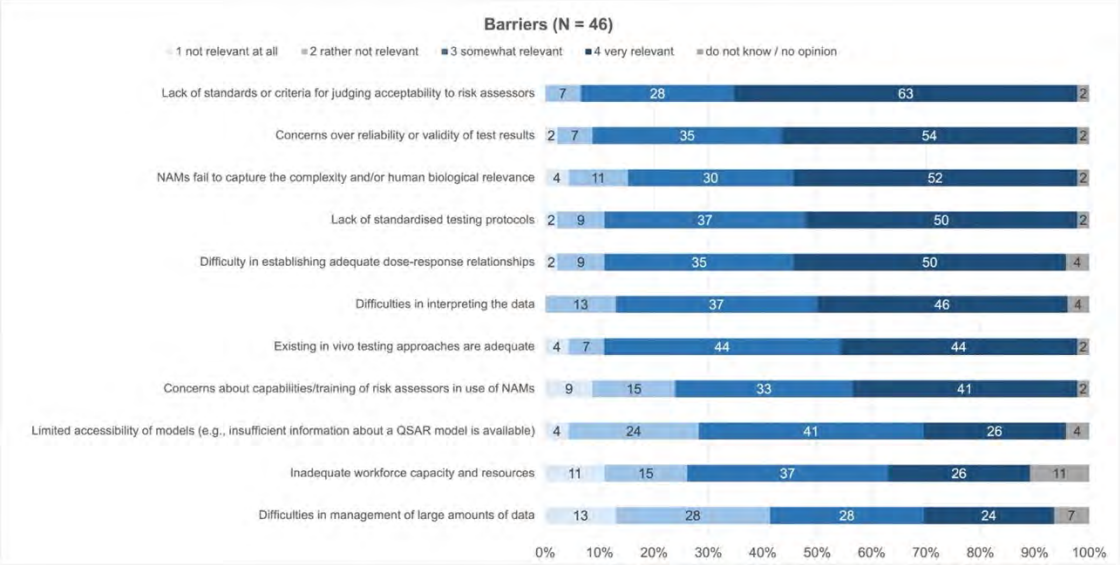


Figure 3. Barriers of the use and regulatory acceptance of NAMs (N = 46, number of participants is lower due to dropouts).

Figure 4 shows that all drivers were considered somewhat or very relevant by most participants (78–100%). Harmonizing protocols and standardizing NAMs reached full agreement (100%), while exploring new toxicity endpoints and accelerating individual chemical assessments were viewed as slightly less relevant (by 18–20%).

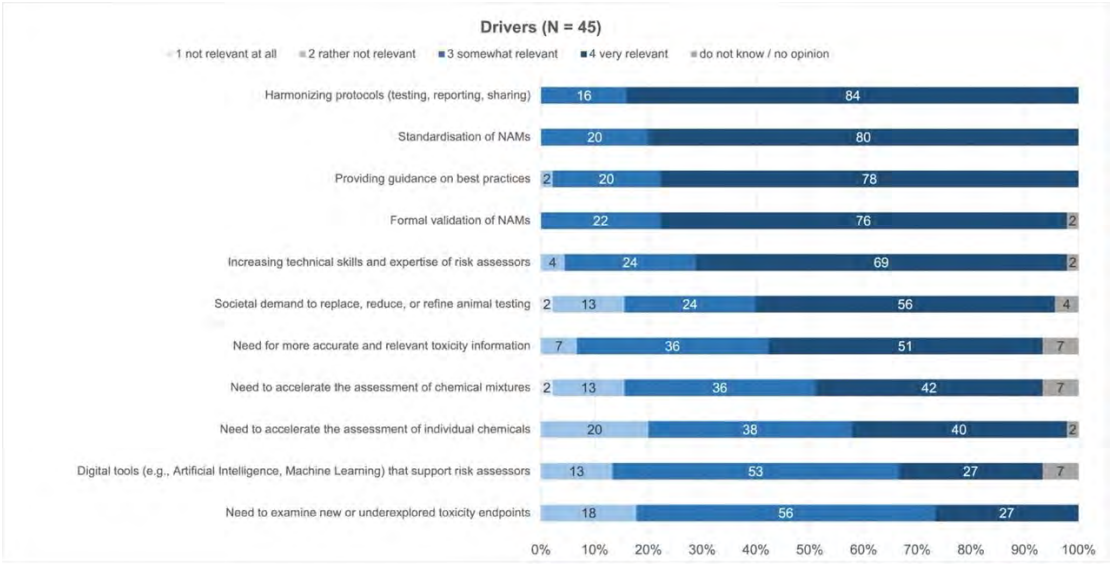


Figure 4. Drivers of the use and regulatory acceptance of NAMs (N = 45, number of participants is lower due to dropouts).

C. Preferences and views of risk assessors

In the final survey section, participants shared personal views and preferences that may influence NAM use and acceptance. As shown in Figure 5, respondents placed greatest importance on formal regulatory acceptance and clear guidance. Characterizing uncertainty and adherence to Good Laboratory Practice were also valued, though opinions were divided on the need for formal validation beyond scientific validity.

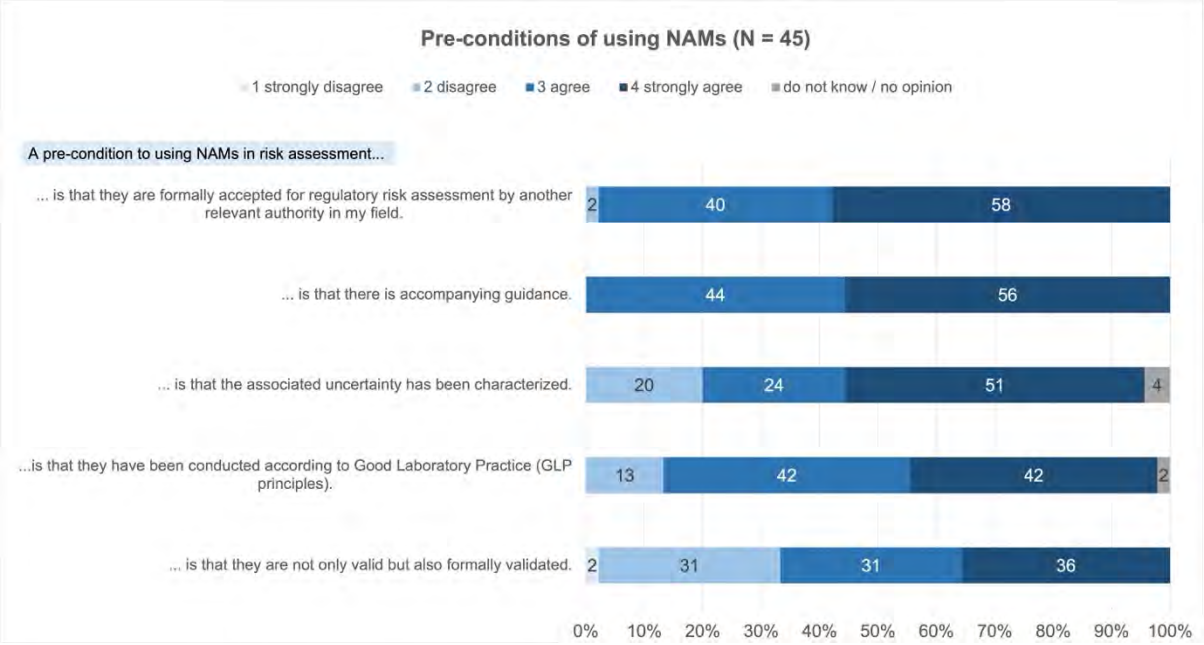


Figure 5. Pre-conditions of using NAMs (N = 45, number of participants is lower due to dropouts).

Participants rated how various stakeholders influence NAM adoption (1 = hinder to 5 = drive). As shown in Figure 6, academia was viewed as the main driver, followed by industry, NGOs/NPOs, and scientific committees. Opinions on regulatory authorities were mixed, with about 40% seeing them as hindering NAM use, while consultants/freelancers were generally viewed as having little influence.

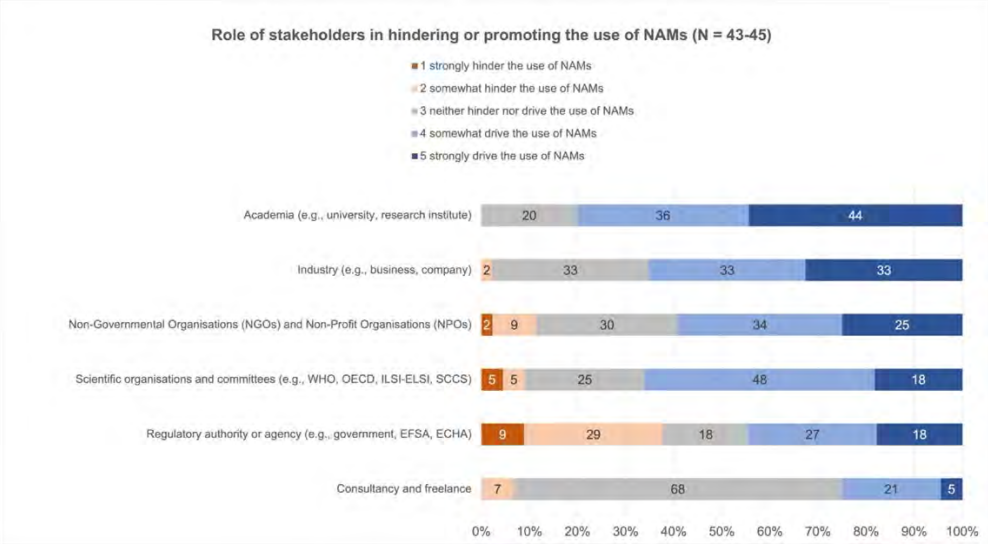


Figure 6. Role of different stakeholders in hindering or promoting NAMs (N = 43-45, number of participants is lower due to dropout and missing responses).

Participants were asked how free they felt in conducting risk assessments (Figure 7). Responses indicated a moderate level of restriction, mainly due to legal requirements, strict guidance, and resistance to non-traditional methods, rather than time or capacity limits. Views varied on having freedom to choose data and methods.

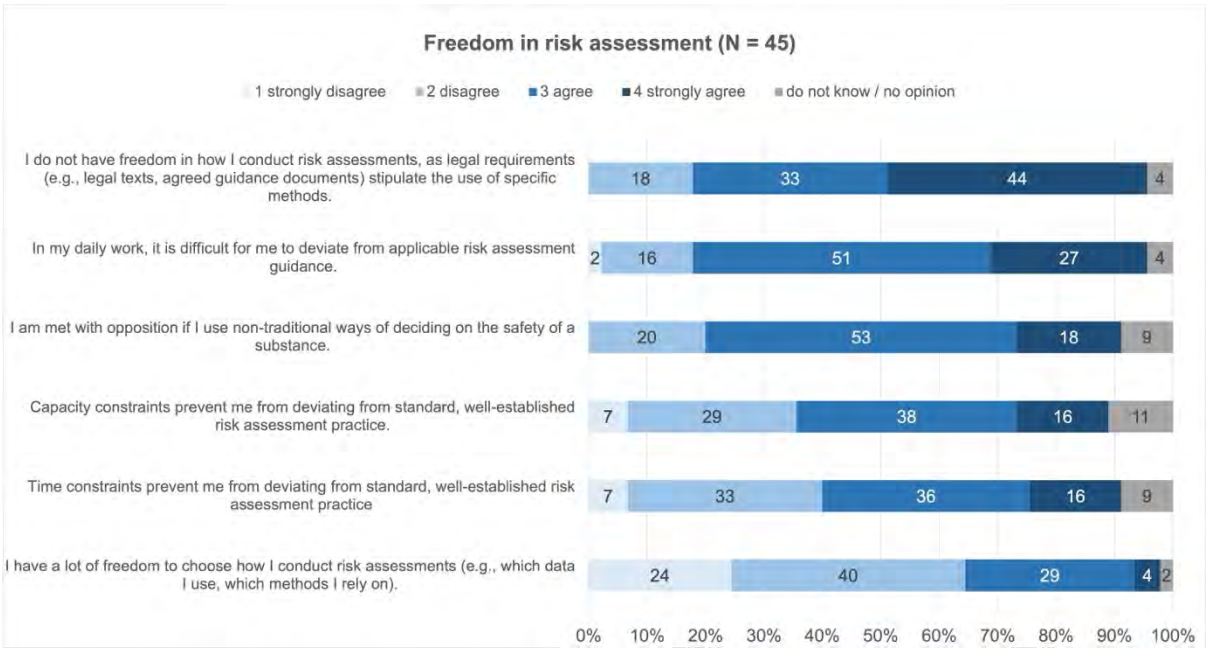


Figure 7. Freedom in risk assessment (N = 45, number of participants is lower due to dropouts).

Common assumptions about risk assessment identified in interviews were tested for representativeness (Figure 8). Responses were highly diverse: opinions were split on whether a gold standard exists, and many had no clear view. Participants showed greater disagreement with the ideas that NAMs should remain only supportive or that animal testing is the gold standard, while views on uncertainty in NAMs vs. animal studies were mixed.

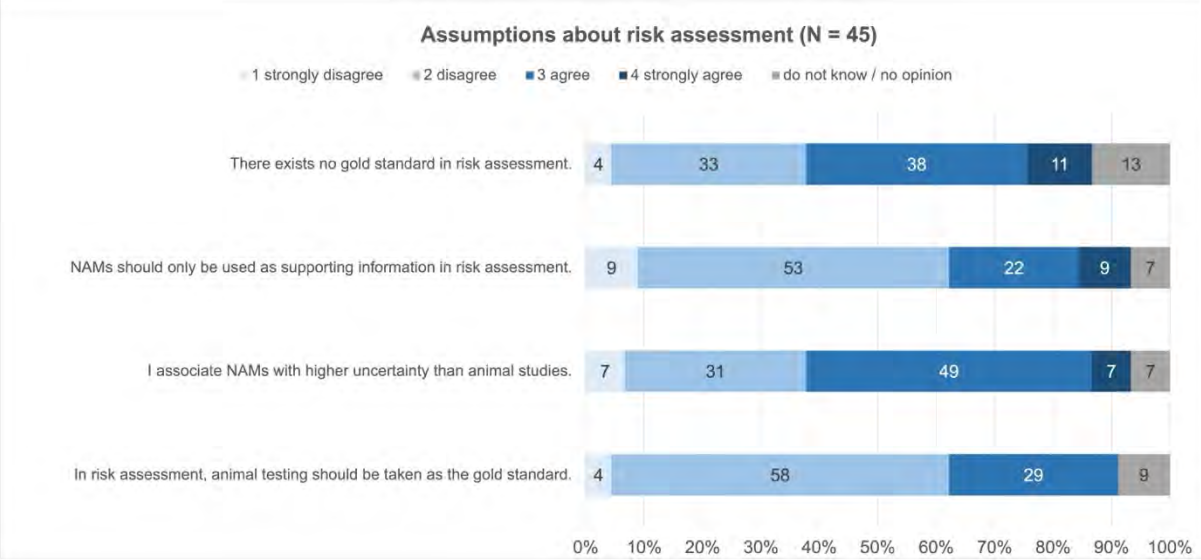


Figure 8. Assumptions of risk assessment (N = 45, number of participants is lower due to dropouts).

Finally, participants evaluated future conditions for risk assessment (Figure 9). There was broad agreement that risk assessment must evolve, requiring legislative change to enable NAM implementation, while remaining central to human and environmental safety. Opinions were mixed on digitalization (e.g., artificial intelligence), and many disagreed that NGRA is incompatible with animal testing or that a zero-pollution environment is achievable.

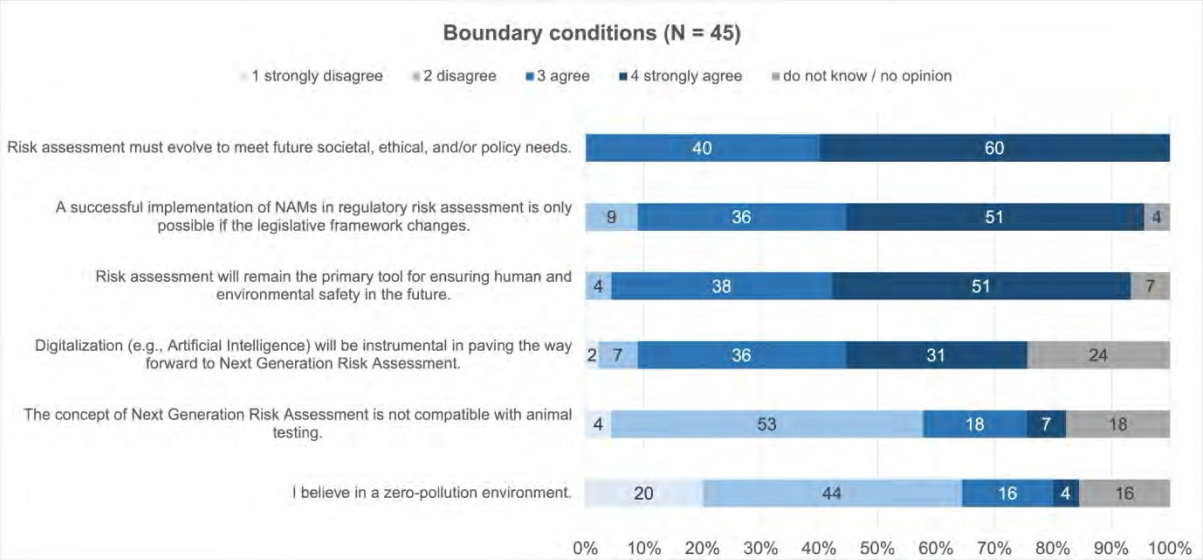


Figure 9. Boundary conditions (N = 45, number of participants is lower due to dropouts).

Textbox 3. Online survey with European risk assessors

The same online survey was distributed among European risk assessors under PARC, yielding N = 325 respondents (222 human health, 103 environmental). The larger sample enabled additional descriptive and multivariate analyses (Bearth et al., 2024b; Bearth et al., 2025a).

Patterns of familiarity and use closely matched the Swiss results: similar methods were well known or rarely used. A key difference was that Swiss participants were significantly more familiar with and used QSAR and PBK modelling more often ($p < 0.001$).

Across Europe, NAMs were most frequently applied for hazard identification/characterization and risk characterization (notably in vitro assays, read-across, QSARs, DA), and less often for screening or exposure assessment.

Open-ended responses on barriers and drivers were thematically coded at the European level (see PARC outputs). In the closed-format items, Swiss and European participants showed very similar patterns, with no significant differences in views or preferences.

6 TECHNICAL REPORT D2: SUMMARY OF THE GAPS & NEEDS ANALYSIS, BARRIERS, OPPORTUNITIES, DRIVERS FOR INTEGRATION AND USE OF NAMS ACROSS SECTORIAL FRAMEWORKS

6.1 COMMON BARRIERS FOR THE USE OF NAMS IN REGULATORY RISK ASSESSMENT ACROSS CHEMICAL SECTORS

Animals and NAMs both aim to ensure chemical safety, but NAMs offer advantages in scope, precision, efficiency, and human relevance, while reducing animal use. They can detect early or subtle toxic effects and cover additional endpoints beyond traditional tests. However, despite these benefits, regulatory acceptance and validation remain key challenges across all chemical sectors.

6.1.1 LEGAL BARRIERS

To advance NGRA globally, legally binding frameworks are needed to define how NAM data can complement or replace traditional animal data. Current SIRs rely on apical animal endpoints, while mechanistic NAM data do not fit existing schemes. Critics view animal tests as tangible but question their human relevance, describing them as “black boxes.” In contrast, human-based in vitro NAMs provide more mechanistic and relevant insights.

A. Absence of legal frameworks

Lack of clear legal guidance creates uncertainty for both regulators and industry. Transitions to NAM-based NGRA must align with existing structures to ensure scientific and legal compatibility (see OECD “Mutual Acceptance of Data”). Current initiatives (Ball et al., 2022; van der Zalm et al., 2022; US FDA, 2019–2023) are non-binding. The EU’s 2013 cosmetics testing ban illustrates the challenge - no new ingredients have since entered the market.

B. Lack of harmonization

Uneven global acceptance limits NAM uptake and creates trade barriers. While REACH remains rigid, Toxic Substances Control Act (TSCA) in the U.S. is more flexible; EPA and US FDA already accept DAs for multiple sectors, whereas Europe limits their use to OECD TG 497 and TG 467 (Schmeisser et al., 2023).

C. Outdated Standard Information Requirements (SIRs)

Legislation must update SIRs to reflect mechanistic endpoints from NAMs (e.g., gene/protein expression, oxidative stress) rather than organism-level outcomes (Schmeisser et al., 2023). Case studies coordinated by OECD and initiatives by the SCCS Methodology Working Group (Rogiers et al., 2022; SCCS, 2023) are paving the way. An NGRA workshop will be held on 10 December 2024 in Brussels.

6.1.2 TECHNICAL BARRIERS

The intended use of a NAM determines the level of validation required. Users may tolerate higher uncertainty when data are scarce or the regulatory impact is low. Nonetheless, validation remains essential—to ensure regulatory confidence in reliability and relevance, and to enable globally harmonized standards (OECD TGs, ICH TGs) and Mutual Acceptance of Data (MAD) under the 3Rs principle (“tested once, accepted everywhere”).

Therefore, the process of validation needs to undergo a paradigm change from universal validation to targeted approaches to allow a faster validation of NAMs that can be accepted by regulatory authorities (Schmeisser et al., 2023).

- **Systemic and chronic toxicity:** NAMs face limitations due to their lack of organismal complexity, toxicokinetic context, and treatment duration, making repeated-dose or systemic toxicity difficult to assess. Regulators remain cautious about relying solely on mechanistic molecular initiating events (MIE)/KE data to predict apical outcomes (Schmeisser et al., 2023).

- **Applicability domain:** Physicochemical properties can compromise in vitro assay performance - including volatility, lipophilicity, solubility, instability, protein binding,

reactivity, size, redox behaviour, pKa, and aggregation. All of which can distort exposure, bioavailability, or assay readouts.

6.1.3 HUMAN AND SYSTEMS-LEVEL BARRIERS

NAMs are inherently multidisciplinary, spanning fields from cell biology to bioinformatics, and their growing complexity poses challenges for regulators in both understanding and resources. Such can be described by the following challenges:

- **Silos and lack of dialogue:**

  Academic and regulatory goals often diverge. Academia focuses on innovation and publication, while regulators need standardized, usable data. This leads to fragmentation and limited communication. Improved dialogue and structured reporting frameworks (e.g., Carnesecchi et al., 2023; OECD, 2018) are essential for successful translation of NAMs into regulation.

- **Lack of capabilities and scepticism:**

  Many regulators are trained in animal-based toxicology and remain unfamiliar or sceptical of NAM technologies. Continuous training and collaboration between developers and risk assessors are needed to shift from apical effect observation to mechanistic prediction (Schmeisser et al., 2023). Ongoing EU and US initiatives (PARC, EU-ToxRisk, PANORAMIX, EURION, ASPIS, and EURL ECVAM) as well as EPA training programs, have already trained thousands of scientists in NAMs (e.g., 15,000+ learners on Coursera, ONTOX project, 2025).

- **Societal expectations:**

  Regulators are cautious that shifting from animal tests could undermine public trust in safety decisions. Incorporating social science insights on societal expectations (Bearth et al., 2024a, b; Ormandy et al., 2012) and promoting stakeholder engagement can strengthen both decision-making and risk communication.

6.1.4 ECONOMIC BARRIERS

NAMs are costly to develop and validate. Validation reflects their scientific and regulatory complexity and the need to ensure reliability and acceptance. Depending on the method, lab costs alone range from €200,000–500,000 (OECD, 2024). These studies demand substantial time, funding, and specialized equipment. With its international, multidisciplinary network, SCAHT could help coordinate and streamline such validation efforts.

6.2 FIT FOR PURPOSE - NAM VALIDATION REQUIREMENTS

The principle of being "fit for purpose" means that a NAM should be appropriately validated for the specific use or application it is intended for (Table 5). This involves considering factors such as the intended regulatory context, the type of chemical being assessed, the potential impact on human health or the environment, and the level of confidence needed in the results.

- Potential uses of NAMs are listed in Table 5.
- A series of questions relevant to determining the fitness for purpose of a NAM for use in a specific regulatory context provided by van der Zalm et al., (2022) are listed in Table 6.
- Crofton et al., (2011) also provide definitions for important terms that describe test systems and a series of recommendations to assist in the transition of methods from early development stages to use in screening and validation efforts Table 7.

Table 5: Different use contexts for New Approach Methods

Content	Explanation
Research & development	NAMs can be used in basic research, applied research and product development as well as in the regulatory settings such as classification and labelling for registration and human health risk assessment for marketing authorization.
High throughput screening Mode of action Modelling	NAMs can be used in HTS programs to quickly assess the toxic potential of chemicals. In research and development, they can be used to investigate the biochemical, physiological, and pathological changes induced by exposure to chemicals and to elucidate their underlying mechanisms of toxicity. NAMs can also be used to model human health effects and predict the potential risks to human populations.
3Rs in the regulatory environment	In the regulatory environment, NAMs can be used in various contexts. In risk assessment, NAMs can be used to replace (e.g. DA skin sensitization instead of LNNA), reduce (grouping of chemicals, read-across, waiver) and refine animal-centric approaches for hazard identification e.g. for classification and labelling, dose response assessment e.g. for definition of threshold values and risk assessment.
Defined approaches Integrated Approach to Testing and Assessment	They can be used as stand-alone decision-making tool or be part of a DA to testing and assessment that consists of a fixed data interpretation procedure (DIP) applied to data generated with a defined set of information sources to derive a result that can either be used on its own (e.g. OECD DA skin sensitization) or together with other information sources within an IATA that combines multiple sources of information to conclude on the toxicity of chemicals.
Weight of evidence	NAMs can provide supporting evidence as part of a WoE approach that integrates the various types of scientific information available to assess the strength of the relationship between a hazardous chemical and a toxicological endpoint. Thus, NAMs can be used to address uncertainties in the risk assessment with the available data.
Tiered testing strategies	NAM s can be part of a structured approach to evaluating the potential risks consisting of multiple levels of testing, with each tier becoming progressively more complex and resource-intensive (screening tests, targeted tests, comprehensive tests).
Prioritization	In risk management NAMs can be used for the prioritization of risk-mitigation measurements or for the grouping of chemicals.

In essence, recognizing that NAM validation requirements may vary by use context emphasizes the need for flexibility and adaptability in the validation process. Different regulatory or scientific contexts may require different levels of validation for these new methods. A regulatory agency might require extensive validation for NAMs intended to replace a traditional animal test for assessing the toxicity of a chemical, while a research application might accept a less validated method for exploratory purposes.

Table 6: Questions relevant to determining the fitness for purpose of a NAM for use in a specific regulatory. Adopted from van der Zalm et al., 2022.

Context	Related questions
Legal context	Which regulatory statutes are the data from the NAM intended to comply with? US EPA Toxic Substances Control Act US TSCS, EU Regulation on the REACH, others?
Use context	What is the context in which the NAM is intended to be used? What is the regulatory problem you want to solve? Pre-regulatory screening and prioritization? Chemical grouping? Hazard identification? Quantitative risk assessment?
NAM Influence	How will the NAM be used? As a stand-alone to drive decision making? As part of a DA, IATA, or WoE to provide supporting evidence?
NAM performance	Is the information provided sufficient to address the regulatory endpoints of interest? What is the relation between the information measured by the NAM and the regulatory endpoints being addressed (e.g. AOP, MIE, KE, KER). Is the level of performance, including the level of uncertainty, acceptable?
Applicability	What is the chemical applicability domain? How many chemicals have been tested? What is the biological applicability domain? Which biological processes are covered? (e.g. DNT-IVB covering formation, migration, differentiation, axon outgrowth, synaptogenesis, myelination, functional networks, …) ?
Reliability	Have the NAMs undergone a classic validation process? Have performance criteria been established and agreed? Have acceptance criteria been established and agreed? Is there a Standard Operating Procedure? e.g., what is the max. +/- deviation that is considered to be within the control range; what is the no. of replicates required Is there a harmonized written decision framework? e.g.: How many assays or what potencies prioritize a compound for follow-up? If using IVIVE what is a sufficient Margin of Exposure? What is the “gold standard” to compare against – in vivo or human? How well do in vivo data represent the human situation?
Impact	What are the potential negative regulatory consequences / risks of using NAMs? Underestimation of risk? Overestimation of risk leading to unnecessary restrictions or unnecessary secondary higher tier testing?

Table 7: Overview of the 15 guidance items for developing alternative screening test methods for developmental neurotoxicity. According to Crofton et al., 2011.

	Methods Area	Methods Elements	Description
1	Key event of neurodevelopment	TM, TS, E	Endpoints should model key aspects of neurodevelopment
2	Endpoint measurement	TM, E	Correct and accurate measurement of the endpoint
3	Dynamic range	E	Determination of the extent of measurable change
4	Parametric control	E	Assay parameters that predictably change the endpoint
5	Response characterization	E	Level of change determined to be an effect
6	Concentration	TS, E	Methods must be designed to allow determination of concentration-response
7	Endpoint selectivity	E	Discrimination of the endpoint of concern from non-specific outcomes
8	Endpoint-selective controls	E	Chemicals known to reliably and consistently alter the endpoint at a mechanistic level.
9	Training set chemicals	TM, E	Goal is proof-of-concept that the test method can rapidly and efficiently screen moderate numbers of chemicals. Should include chemicals known to reliably elicit a response, or no response, based on in vitro findings.
10	Testing set chemicals	TM, TS, E	Goal is to demonstrate ability to test large number of chemicals. Should include chemicals known to affect, and lack effects, on in vivo developmental neurotoxicity endpoints.
11	Specificity and Sensitivity	TM, E	Analysis to determine ability to correctly differentiate active and non-active chemicals
12	High throughput	TS, E	Test system and endpoint should be amenable to automation
13	Documentation	TM	Full and published documentation of the test method
14	Transferability	TS, E	Resources for use should be available for any laboratory
15	Data sharing	Data	Open access databases are highly desirable.

TM: Test method, TS: Test system, E: Endpoint

6.3 OVERARCHING ACTIVITIES TO ADVANCE THE IMPLEMENTATION OF NAMS

6.3.1 INTERNATIONAL COLLABORATION

The development and regulatory implementation of NAMs depends on close collaboration across stakeholders including regulatory offices, international agencies, industry, academia,

and NGOs. Key international drivers include the OECD and the International Cooperation on Alternative Test Methods (ICATM). The OECD's 38 member countries publish test guidelines, many describing non-animal methods accepted for chemical safety testing, and develop frameworks such as IATA, AOPs, and Guidance Document 34 (GD 34) to support their regulatory uptake.

ICATM brings together ICCVAM (US), Health Canada, EURL ECVAM (EU), JaCVAM (Japan), KoCVAM (Korea), BraCVAM (Brazil), Chinese, and Taiwanese centers to coordinate national and joint promotion of NAMs. Examples include EURL ECVAM's TSAR database, which tracks alternative methods, and US NICEATM's list of NAMs accepted by US agencies. Table 8 lists links to repositories for NAMs.

Table 8: Links to repositories for New Approach Methodologies

#	Links to repositories for New Approach Methodologies
1	Tracking System for Alternative Methods (TSAR): EURL ECVAM provides an overview of non-animal methods that have been proposed for regulatory safety or efficacy testing of chemicals or biological agents. Their TSAR, indicates the stages methods have reached in terms of acceptance as a recognised test method for use in various sectors together with a summary description. Where available, TSAR also includes relevant records and documents associated with a method linked to the different steps of the entire process: submission, validation, peer-review, recommendations and regulatory acceptance including international standards represented in the tracking system (Stucki et al., 2022).
2	A list of NAMs accepted by US agencies can be found on the website of the US National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), which supports ICCVAM's work.
3	Another US List of Alternative Test Methods and Strategies (or New Approach Methodologies [NAMs]) from 2021 can be found here: https://www.epa.gov/sites/default/files/2021-02/documents/nams_list_second_update_2-4-21_final.pdf
4	Screening data generated with an extensive battery of in vitro assays for an impressive number of chemicals under ToxCast (https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data) and Tox21 (https://tox21.gov/resources/) are widely used and appreciated by the regulatory community, e.g., Endocrine Disruptor Screening Program (EDSP) data for mechanistic considerations within the European pesticides regulation (Schmeisser et al., 2023).

6.3.2 INTERNATIONAL AND NATIONAL GUIDANCE AND ROADMAPS

Numerous national and sector-specific guidance documents, roadmaps, and frameworks aim to facilitate NAM implementation, yet navigating them remains challenging. Many are too general or too case-specific, lack actionable detail and harmonization, and often overlook uncertainty analysis (Rogiers et al., 2022). The most persistent barrier remains the absence of clear legal and regulatory integration pathways.

Tables 9 and 10 (adapted from Stucki et al., 2022) summarize agency guidelines, successful NAM applications, and collaborations that build confidence in their regulatory use. Key initiatives include:

- **ICCVAM Strategic Roadmap:** Guidance for U.S. agencies and stakeholders adopting NAMs.
- **US EPA Roadmaps and Work Plans:** Based on the NRC report *Toxicity Testing in the 21st Century* (NRC, 2007; US EPA, 2009, 2021), promoting NAMs for chemical and pesticide testing.
- **EU Directive 2010/63/EU** (EU, 2010) and the **Chemicals Strategy for Sustainability** (European Commission, 2020): Mandate use of non-animal methods where possible and support NAM innovation. The Commission roadmap will define milestones toward phasing out animal testing.
- **EFSA 2027 Strategy:** Commits to integrating NAMs into regulatory risk assessment, with a roadmap and multiannual action plan to reduce animal testing and strengthen use of IATA and read-across (EFSA, 2021; Escher et al., 2022).
- **European Commission:** Currently preparing a European Roadmap towards phasing out animal testing for chemical safety assessment (European Commission, 2023), which is the response to a European Citizens Initiative and which will be published in the first quarter of 2026.

These coordinated efforts are crucial for achieving legal clarity, regulatory harmonization, and global uptake of NAMs in safety assessment.

Table 9: US, Canada, and EU: industrial chemicals and household products, Strategic plans, guidance and other documentation for implementation of NAMs referenced by Stucki et al., 2022.

Agency	Topic
EPA OPPT	Interim science policy: use of alternative approaches for skin sensitization as a replacement for laboratory animal testing
	Strategic plan to promote the development and implementation of alternative test methods within the TSCA program
	Utility of In Vitro Bioactivity as a Lower Bound Estimate of In Vivo Adverse Effect Levels and in Risk-Based Prioritization
	List of alternative test methods and strategies (or new approach methodologies [NAMs]), Second update: 4 February 2021
	New approach methods work plan, reducing use of animals in chemical testing
	A WoE Approach for Evaluating, in Lieu of Animal Studies, the Potential of a Novel Polysaccharide Polymer to Produce Lung Overload
CPSC	Recommended Procedures Regarding the CPSC's Policy on Animal Testing (16 CFR Part 1500)
	Guidance on Alternative Test Methods and Integrated Testing Approaches
HC HECSB	Fact sheet series: Topics in risk assessment of substances under CEPA
	Guidance document for the notification and testing of new chemicals and polymers
	Canadian regulatory perspective on next generation risk assessments for pest control products and industrial chemicals
	Utility of In Vitro Bioactivity as a Lower Bound Estimate of In Vivo Adverse Effect Levels and in Risk-Based Prioritization
	Science approach documents

ECHA	How to use alternatives to animal testing to fulfil the information requirements for REACH registration
	Read-across assessment framework
	4th report on the use of alternatives to testing on animals for REACH
	Utility of In Vitro Bioactivity as a Lower Bound Estimate of In Vivo Adverse Effect Levels and in Risk-Based Prioritization
	Skin sensitization

CEPA, Canadian Environmental Protection Act; CFR, Code of Federal Regulations; CPSC, Consumer Products Safety Commission; ECHA, European Chemicals Agency; EPA OPPT, Environmental Protection Agency Office of Pollution Prevention and Toxics; HC HECSB, Health Canada Healthy Environments and Consumer Safety Branch; NAM, new approach methodologies; TSCA, Toxic Substances Control Act; WoE, weight-of-evidence.

Table 10: US, Canada, and EU: pesticides and plant protection products, Strategic plans, guidance and other documentation for implementation of NAMs referenced by Stucki et al., 2022

Agency	**Topic**
EPA OPP	Guidance for waiving or bridging of acute and repeat dose toxicity tests for pesticides (for formulations and single-active ingredients)
	Use of an alternate testing framework for classification of eye irritation potential of EPA pesticide products
	Process for evaluating & implementing alternative approaches to traditional in vivo acute toxicity studies for FIFRA regulatory use
	Interim science policy: Use of alternative approaches for skin sensitization as a replacement for laboratory animal testing
	Recommendation on test readiness criteria for new approach methods in toxicology: Exemplified for DNT
	Utility of In Vitro Bioactivity as a Lower Bound Estimate of In Vivo Adverse Effect Levels and in Risk-Based Prioritization

	New approach methods work plan, reducing use of animals in chemical testing
	Retrospective analysis of the dermal absorption “triple pack” data
HC HECSB	Performance of the GHS Mixtures Equation for Predicting Acute Oral Toxicity
	Integration of toxicodynamic and toxicokinetic new approach methods into a WoE analysis for pesticide DNT assessment
	ReCAAP: A reporting framework to support a WoE safety assessment without long-term rodent bioassays
HC PMRA	Guidance for waiving or bridging of mammalian acute toxicity tests for pesticides, Acute Dermal Toxicity Study Waiver
	PMRA’s 2016–2021 strategic plan
	Canadian regulatory perspective on next generation risk assessments for pest control products and industrial chemicals
	Guidance for developing datasets for conventional pest control product applications
	ReCAAP: A reporting framework to support a WoE safety assessment without long-term rodent bioassays
EFSA	Guidance on dermal absorption
	OECD/EFSA workshop on DNT: The use of non-animal test methods for regulatory purposes
	Reconnecting exposure, toxicokinetics and toxicity in food safety: OpenFoodTox and TKPlate for human health, animal health and ecological risk assessment
	Recommendation on test readiness criteria for new approach methods in toxicology: Exemplified for DNT

	Workshop on in vitro comparative metabolism studies in regulatory pesticide risk assessment
	Advancing human health risk assessment
	Utility of In Vitro Bioactivity as a Lower Bound Estimate of In Vivo Adverse Effect Levels and in Risk-Based Prioritization
	Development of IATA case studies on DNT risk assessment
	EFSA Strategy 2027
	Development of a Roadmap for Action on New Approach Methodologies in Risk Assessment

EFSA, European Food Safety Authority; EPA OPP, Environmental Protection Agency Office of Pesticide Programs; FIFRA, Federal Insecticide, Fungicide, and Rodenticide Act; HC PMRA, Health Canada Pest Management Regulatory Agency; NAM, new approach methodologies. ReCAAP, Rethinking Chronic toxicity and Carcinogenicity Assessment for Agrochemicals Project.

The following section provides concise summaries of key publications from the past two years that have advanced the field of NAMs in toxicology and chemical risk assessment, highlighting how these approaches are transforming the discipline from animal-centric methods to mechanistic, human-relevant hazard assessment.

Schmeisser et al. (2023) present the current state and potential of NAMs, including QSAR, high-throughput screening, omics, organoids, microphysiological systems, and artificial intelligence. The publication emphasizes the ability of these approaches to enhance efficiency, biological relevance, and ethical standards in chemical safety assessment, and outlines the need of strategies for their systematic integration into regulatory decision-making.

Browne et al. (2024), focused on redefining “adverse effect” interpretation. It contributes to NAMs by arguing that alternative methods should not be benchmarked against non-specific or misleading animal endpoints, proposing criteria for adversity grounded in biological mechanism.

Longhin et al. (2024) focus was on improving hazard assessment strategies for nanomaterials, whose unique properties challenge current regulatory approaches. The paper contributes to NAMs by combining physicochemical profiling, in vitro assays, and in silico modelling to show how nanoform-specific behavior can be predicted without extensive animal testing. It demonstrates a NAM-compatible and nano-tailored assessment strategies essential for NGRA.

Basu et al. (2025) is a special issue focused on reviewing the landscape of NAMs for ecotoxicology and how they can be used to reduce reliance on traditional animal-based environmental testing. Its relevance is high by summarizing and promoting NAMs for ecotoxicology, supporting a shift toward more ethical, efficient, and human- and environment-relevant testing strategies that can better inform regulatory and environmental risk decisions.

Deepika et al. (2025) review how NAMs can improve the human relevance and efficiency of chemical risk assessment. It contributes by detailing how in-vitro systems, PBK models, AOP-based frameworks, read-across and omics and computational approaches can be integrated into modernized assessment workflows. Its demonstrates that NAM-driven approaches can support regulatory decisions while reducing reliance on traditional animal tests.

Together, these papers illustrate the ongoing scientific and regulatory shift toward more mechanistic, predictive, and animal-free safety evaluation frameworks.

In the past years, US FDA (FDA, 2025) and US National Institutes of Health (US NIH, 2025) have published strategic roadmaps advancing the adoption of NAMs for chemical safety assessment. Meanwhile, the European Union is set to unveil its own roadmap for the progressive phase-out of animal testing, with expectations for its release in early 2026. Two recent publications, Cronin et al. (2025) and Walder et al. (2025), provide a comprehensive view of the EU roadmap for phasing out animal testing in chemical safety assessments. The outcomes from multi-stakeholder discussions aimed at accelerating the development, validation, and regulatory integration of NAMs was reported.

Following these developments, ECHA's Key Areas of Regulatory Challenge report (ECHA, 2025) underscores that advancing NAMs to replace animal testing is an urgent regulatory priority, with particular emphasis on neurotoxicity, immunotoxicity and endocrine disruption. The publication highlights that read-across, in vitro and in silico ADME approaches, and PBK models could serve as suitable substitutes for generating in vivo data under REACH. The report also stresses the need for validated NAMs to support complex areas such as fish toxicity, carcinogenicity and the assessment of micro- and nano-sized materials.

By linking scientific progress with regulatory priorities, this section provides a perspective on the pathway toward next-generation risk assessment.

6.4 CASE STUDIES ON THE USE OF NAMS TO MEET REGULATORY REQUIREMENTS FOR THE ASSESSMENT OF CHEMICALS

Several NAMs are already fit for regulatory use for endpoints such as local toxicity (skin sensitization, irritation, corrosion, phototoxicity), dermal absorption, genotoxicity, and pyrogenicity. Their value is increasingly recognized by US EPA, US FDA, EFSA, and the OECD, and they are integrated into IATA frameworks (Schmeisser et al., 2023). NAMs are also used for chemical screening and prioritization (Luijten et al., 2022), read-across support (Ball et al., 2020), and data gap filling (Escher et al., 2019; Rovida et al., 2020; van der Stel et al., 2021).

However, complex endpoints such as repeated-dose, reproductive toxicity, and carcinogenicity remain challenging. Replicating an OECD TG 408 sub-chronic study via NAMs would require full coverage of organs, exposure duration, and a reliable PoD, which is potentially achievable only through battery approaches targeting AOP-defined key events. Yet, under the UN GHS and EU CLP Regulation (EC 1272/2008), classification and labelling still depend on in vivo or human data, making NAM-only submissions nearly impossible (Schmeisser et al., 2023).

While US and EU regulations provide some flexibility, implementation differs by sector (Stucki et al., 2022). Under REACH, registrants must compile all relevant data, i.e. human, animal, and NAM-based, including (Q)SARs, read-across, and in vitro results. OECD GD 34 defines pre-validation criteria for using in vitro data, though negative results still require in vivo

confirmation. Amendments to REACH in 2016–2017 now mandate NAM use for certain endpoints, resulting in a threefold increase for skin corrosion/irritation, fourfold for eye irritation, and over 20-fold for skin sensitization (ECHA, 2020).

The following 14 case studies available so far are extensively described in the original deliverable D2 (report from November 2024, 6.4.1 – 6.4.14) and will in this summary report just be listed.

1. Eye corrosion/irritation testing using DA (US EPA)
2. Dermal absorption Testing using in vitro human skin (US EPA)
3. Acute oral toxicity prediction using in silico models (US EPA)
4. Inhalation Risk Assessment using in vitro and in silico models (US EPA)
5. Waiving subchronic inhalation toxicity Testing using in-silico modelling (US EPA)
6. Waiving in vivo genotoxicity testing using in vitro testing and read-across (ECHA)
7. Developmental Neurotoxicity In Vitro Testing Battery (DNT IVB)
8. DNT assessment of individual organophosphates (OP) using DNT-IVB (US EPA)
9. DNT assessment of acephate / and its metabolite methamidophos using a WoE-approach with DNT-IVB data (US EPA)
10. In vitro data to waive DNT in vivo testing for L-glufosinate isomers (US EPA)
11. in vitro neurotoxicity test triggered further testing for the Fungicide dicloran (US EPA)
12. IATAS for DNT testing of flufenacet and Deltamethrin (EFSA)
13. AOP-based IATA to assess Parkinsonian motor deficits (EFSA)
14. NAMs in the Endocrine Disruptor Screening Program (US EPA)

6.5 GAPS AND NEEDS ANALYSIS

Despite major advances, several toxicological endpoints remain poorly addressed by current NAMs, though they are critical for human health risk assessment:

- **Long-term effects:** Chronic and carcinogenic outcomes as well as complex endpoints, like developmental and reproductive toxicity, remain difficult to predict from short-term or acute NAMs.

- **Immunotoxicity:** The immune system's complexity hampers modelling of immune suppression or overstimulation.

- **Endocrine disruption:** With the implementation of the new endocrine-disruptor (ED) hazard class under CLP (Commission Delegated Regulation (EU) 2023/707), substances may now be formally classified for ED with weight of evidence support of NAMs. However, guidance and validated test methods particularly for non-EATS mechanisms remain limited, and thus certain endocrine modes of action may still pose challenges.

- **Cumulative effects:** Real-world exposures involve chemical mixtures with additive or synergistic effects rarely captured by single-chemical NAMs.

Progress will require integrated approaches like IATAs or DA, in combination with the AOP framework, combining in vitro assays, omics, computational and high-throughput methods with advanced exposure models and data analytics, supported by cross-sector collaboration to refine and validate NAMs for these complex endpoints.

Pharmaceuticals

Applicants for new drugs and biologics must provide nonclinical pharmacology and toxicology data demonstrating that clinical trials are reasonably safe. These data (typically from animal or in vitro studies) guide development and regulatory review, as outlined in US FDA and ICH guidance, which allows flexible, nonbinding approaches if regulatory standards are met. While the current paradigm performs well for many endpoints, its predictive value for others (such as carcinogenicity, immunosuppression, and drug-induced liver injury) remains limited. Table 11 summarizes key opportunities and challenges for refining these approaches.

Table 11: An overview of current key challenges and needs in nonclinical safety assessments supporting development of human pharmaceuticals (Avila et al., 2020).

Assessment	Key Issues Addressed	Key Needs
Safety Pharmacology	Identifies potential adverse pharmacological effects of the drug in vitro and/or in vivo	1_Refine approach to improve identification of proarrhythmic risk while reducing attrition of potentially useful drugs from clinical testing
	Identifies specific parameters to monitor more closely in clinical trials	2_Refine Tier 1 neurological screens to achieve more quantitative and objective measures
		3_Standardize Tier 1 protocols and statistical approach to enable cross-laboratory comparisons
		4_Improve Tier 2 testing of higher cognitive functions and specificity of sensory tests
General Toxicity	Estimates safe "first in human" (FIH) starting dose	5_Improve risk identification for rare and idiosyncratic toxicities
	Estimates safe maximum doses in early clinical trials	6_Refine cardiovascular assessments to include functional endpoints
	Identifies possible consequences of chronic exposure	7_Improve approach to evaluating human relevancy of toxicity findings in animals
	Identifies specific parameters to monitor more closely in clinical trials	8_Identify best contexts-of-use for animal models of disease for toxicology testing
		9_Develop more human-relevant approaches to assessing risk of immune-directed therapies
Carcinogenicity	Predicts long-term risks that are difficult to assess or are unethical to assess in humans	10_Develop approaches more applicable to human biology and that provide dose response data based on anticipated human exposure and mechanism.
Developmental and Reproductive Toxicity	Predicts risks that are difficult to assess or are unethical to assess in humans	11_Develop sensitive alternatives to detect potential teratogenicity earlier to facilitate clinical trials.
	Identifies risks for special populations	12_Develop sensitive alternatives for drug classes not tolerated by pregnant animals, such that the ability to conduct in vivo developmental and reproductive toxicity studies is limited

	Identifies specific parameters to monitor more closely in clinical trials	13_Develop validated non-animal methods for assessing human skin sensitization of mixtures
	Allows the mechanistic understanding of an adverse biological change observed in animals or humans	

Despite these challenges, NAMs are widely used in pharma, supported by regulatory flexibility and access to human pharmacokinetic data. This enables quantitative IVIVE and better correlation with histopathology and clinical chemistry findings.

6.6 CAN THE COSMETICS REGULATION SERVE AS A ROLE MODEL FOR THE IMPLEMENTATION OF NAMS?

Since 2013, the EU Cosmetics Regulation has banned animal testing for cosmetic products and ingredients, requiring all safety data to be derived from animal-free NAMs. This has made cosmetics a test case for innovation in regulatory toxicology.

The ban has encouraged broader use of in chemico, in silico, QSAR, read-across, PBK models, and in vitro assays, integrated in a WoE approach alongside pre-ban animal data and limited human evidence. Non-animal methods are now accepted for local toxicity (e.g., skin and eye irritation), but validated alternatives for systemic endpoints, such as chronic, reproductive, or carcinogenic toxicity, are still lacking.

Although pre-ban animal data and results from other sectors (e.g., food, pharma, biocides) may legally be used, few companies rely on them, reflecting strong consumer demand for cruelty-free products (Rogiers et al., 2020; Bearth et al., 2024a).

Despite substantial scientific progress, and the associated ethical benefits, in cosmetics testing and NGRA, certain regulatory constraints remain. NGRA is not yet mature enough to support definitive safety conclusions based solely on NAMs. In other words, a fully NAM-based dossier can currently not guarantee safety for systemic effects. This is reflected in the fact that no new ingredients have been introduced exclusively for cosmetic use since the ban.

6.7 REGULATORY FLEXIBILITY OF RELEVANT AUTHORITIES IN SWITZERLAND

There appears to be limited regulatory flexibility from Swiss authorities regarding the alternative use of NAMs in chemical risk assessment for marketing authorization as data requirements between Switzerland and the EU are largely harmonized.

The Federal Council aligned Swiss chemical legislation with EU REACH, CLP, and related frameworks to:

1. avoid trade barriers,
2. ensure protection of health and environment,
3. keep pace with technical progress, and
4. reduce animal testing (Anmeldestelle, 2024).

The Chemicals Ordinance (ChemO) (as of Sept 2024) mirrors REACH, CLP, and the ECHA SVHC list.

The Biocidal Products Ordinance (OBP) follows the EU BPR, with mutual recognition under the EU–Swiss MRA, though GMO-based products are excluded.

The Pesticide Ordinance (PSMV) closely matches EU Regulation 1107/2009. A full revision aims to accelerate approvals by automatically recognizing EU decisions.

Switzerland also applies the OECD MAD and UN GHS/CLP systems.

At the same time, Switzerland's active participation in international expert bodies (e.g., the OECD), its support for European research projects (e.g., PARC), and the commissioning of projects to national research infrastructures (e.g., SCAHT) provide opportunities to help shape the development and harmonization of new assessment methods and thereby indirectly influence their future application within its own regulatory system.

Moreover, within its existing legal instruments, Switzerland can act independently when robust data or national conditions warrant stricter measures than those applied by its

European neighbours. Although Swiss risk assessments may use NAM data in addition to EU evaluations e.g., to address local environmental conditions, there are very limited validated NAMs yet available for regulatory risk assessment.

6.8 CONCLUSIONS

Chemical safety assessment still relies heavily on animal testing, which raises ethical, technical, and translational concerns. NAMs, including high-throughput screening, omics, organ-on-a-chip, and AI-based models, offer promising, human-relevant alternatives (BfR, 2021; Deepika et al., 2025).

Key technical needs:

- Stronger linkage between mechanistic NAM data and traditional apical endpoints.
- Test batteries covering complex biological processes.
- Standardized protocols and data interpretation procedures (DIPs).
- Including in vitro and in vivo kinetics by quantitative IVIVE.
- Case studies validating sensitivity, specificity, and reproducibility across endpoints.
- Expansion of IATA/DA frameworks beyond current use (e.g., to endocrine, genotoxic, neuro- and developmental toxicity).

Key regulatory needs:

- Guidance documents for integrating NAM data into decision frameworks (e.g., WoE evaluation).
- Context-specific case studies illustrating use in data-poor and data-rich scenarios.
- Inclusion of dedicated NAMs sections in regulatory guidelines.

With its scientific expertise, international collaborations and participation in major research initiatives, SCAHT is strategically positioned to advance NAM implementation in regulatory practice.

6.9 SCAHT ACTION PLAN

The overall goal of the SCAHT is to promote the regulatory implementation of NAMs and AOPs through research, guidance, training, and collaboration. Specifically, the main SCAHT actions for supporting the regulatory acceptance and application of NAMs are summarized below:

1. International expert participation

- Active member of OECD expert groups, including those revising OECD GD 34 on NAM validation and the DNT IVB.
- Contributes to international efforts advancing NGRA and non-animal testing strategies e.g. through the `European Roadmap towards phasing out animal testing for chemical safety assessment`.

2. Training and capacity building

- Delivered five expert webinars (June–Oct 2024) on NAMs and AOPs. Organizing on-site workshop (Nov 2024, Liebefeld) for Swiss regulatory authorities on integrating NAMs into practice (for details see D3 of this report).
- Hosts research retreat sessions fostering dialogue between scientists and regulators. Founded and coordinates the ISTNET-DART network to advance DART NAMs and roadmap development for the EU.

3. AOP research and development

- Funds mechanistic AOP-driven research addressing regulatory endpoints.
- Created a permanent AOP research position and student support line within SCAHT through the AOP_HUB. The Hub enables global collaboration, resource sharing, and mentoring among students and researchers working on AOPs and NAMs.
- Integrates AOP contributions as performance indicators in research evaluation.

4. Set-up of national and international Case Studies using NAMs

- Case studies were identified as key regulatory needs for building trust in the application of NAMs. The SCAHT will set up novel Swiss case studies and participate in international Case Studies e.g. through PARC.

5. National collaborations

- Expanding partnerships with Eawag, Swiss 3R Competence Centre, and Swiss Ecotox Centre.
- Promotes One Health-based, cross-disciplinary research on endocrine disruption, DART, and replacement of animal testing.
- Builds on OECD-recognized success of the DNT IVB and the Eawag fish cell line assay (TG 249).

6. Validation and funding

- Exploring creation of a public–private validation platform for NAMs, coordinated by SCAHT and funded by industry consortia in the future.

7. Education and curricula modernization

- Updating MAS and MSc Toxicology curricula to integrate NAMs, AOPs, NGRA, AI, and bioinformatics thereby ensuring that graduates are equipped for future regulatory and scientific challenges in human health risk assessment.

- SCAHT's unique strength is to combine regulatory expertise, academic research, and international partnerships to bridge science and policy for implementing animal-free, human-relevant safety assessment in Switzerland and beyond.

7 WORKSHOP REPORT D3: ACTION PLAN FOR PRIORITIZATION OF REGULATORY-RELEVANT SCENARIO-BASED CASE STUDIES

Case studies were identified as key technical and regulatory needs for the future implementation of NAMs into the risk assessment process. Therefore, the SCAHT within this project initiated a process for identifying Swiss case studies, which will contribute to the overall regulatory acceptance of NAMs. The process was divided into three phases:

1) Knowledge generation by a webinar series
2) Case study proposals by Swiss agencies
3) Brainstorming and prioritization exercise by an in-person workshop

7.1 WEBINAR SERIES `SCAHT LUNCH LECTURE SERIES ON NEXT GENERATION RISK ASSESSMENT (NGRA)`

In June 2024, SCAHT launched a webinar series on NGRA to introduce key scientific and regulatory advances in this emerging field. The five-part online series brought together renowned experts from EFSA, Unilever, and SCAHT, serving as a preparatory step for a forthcoming workshop: `Promoting the use of NAMs in regulatory human health risk assessment`. Each session focused on a distinct aspect of NGRA covering grounds from the general principles of next generation approaches to the practical application of AOPs in chemical safety assessment. Predrag Kukic (Unilever) opened the series on June 25th with an overview of NGRA (77 participants), followed by Jean Lou Dorne (EFSA) on July 3rd presenting EFSA's open-access TK/TD modelling platform TKPlate 1.0 (74 participants). On August 20th, Iris Mangas (EFSA) explored a bottom-up AOP generation using deltamethrin and developmental neurotoxicity as a case study (67 participants), complemented by Andrea Terron's (EFSA) top-down AOP approach focusing on Parkinson's disease on September 23rd (59 participants). The series concluded on October 2nd with Ellen Fritsche (SCAHT) presenting an `inside-out` perspective on AOP development (37 participants). Overall, the NGRA webinar

series successfully fostered knowledge exchange with the experts and served as informative preparation for the planned workshop. The lectures are summarized in table 12 below.

Table 12: Summary of the 2024 SCAHT Webinar series on NGRA. The webinar recordings are available upon request to the SCAHT.

Date	Speaker	Topic	Attendees
25th June	Predrag Kukic (UNILEVER)	*Introduction to next generation risk assessment*	77
3rd July	Jean Lou Dorne (EFSA)	*TKPLATE 1.0: EFSA's open access platform for toxicokinetic and toxicodynamic modelling*	74
20th August	Iris Mangas (EFSA)	*AOP generation: Bottom-up approach deltamethrin and DNT*	67
23rd September	Andrea Terron (EFSA)	AOP generation: Top-down approach, the Parkinson's Disease case	59
2nd October	Ellen Fritsche (SCAHT)	*AOP generation: Inside-out approach*	37

7.2 CASE STUDY PROPOSALS BY SWISS AGENCIES

After the webinars the SCAHT asked the Swiss agencies to suggest possible case studies, which should be the practical grounds for discussion in the workshop. For aiding with the task, the SCAHT offered additional meetings in small groups with specific agencies upon request. These meetings took place with members of the FOPH (November 5th 2024) and the FSVO (Federal Food Safety Veterinary Office) (September 2024). Following, 7 topics for case studies were sent to the SCAHT and the SCAHT suggested one additional case example. These suggestions are summarized in table 13.

Table 13: Case Study suggestions by Swiss agencies and the SCAHT.

Number (Agency)	CASE STUDY
1 (FSVO)	Exposure to Succinate Dehydrogenase Inhibitors (SDHIs) leading to cancer
2 (FOPH)	Understand the effects/potency of the Bisphenol (BP) A (BPA) substitute BPTMC
3 (FOPH)	Assessment of the DNT effects of metal mixtures
4 (FOPH)	Fabric softeners and skin sensitization
5 (FOPH)	Can we explain the region-specific overrepresentation of Thelarche in Switzerland?
6 (FOPH)	Respiratory Sensitization
7 (FSVO)	Nanocellulose as a food preservative
8 (SCAHT/ MAK comm.)	1,2-dichloropropane as a human carcinogen

An online preparatory workshop took place on October 15th 2024 in preparation for the in-person workshop. Participants represented the SECO, FSVO, FOPH, BAFU and Swissmedic. The workshop format and procedures were discussed.

7.3 WORKSHOP ON `PROMOTING THE USE OF NAMS IN REGULATORY HUMAN HEALTH RISK ASSESSMENT`

SCAHT organized an in-person workshop in Basel on November 11th 2024, which aimed at i) fostering exchange between Swiss regulatory authorities, international experts, international regulatory representatives and SCAHT scientists, ii) deepening the understanding of frameworks and tools used in NGRA and iii) setting up Swiss case studies in a brainstorming exercise to advance the implementation of NAMs in chemical risk assessment. The Swiss case studies should illustrate the practical application, feasibility and added value of NAM-based approaches within real-world regulatory contexts. They are envisioned to serve as tangible examples of innovation in risk assessment, potentially contributing to ongoing international

initiatives such as PARC and supporting broader OECD-level efforts to integrate non-animal testing strategies into NGRA frameworks.

7.3.1 WORKSHOP PROGRAM

The event began with a half-day plenary session, featuring presentations by the speakers from the earlier SCAHT NGRA webinar series, who provided scientific and strategic perspectives on advancing NGRA. These talks were also thought as mental reminders of the past webinars. In the afternoon breakout sessions, participants from the Swiss authorities were randomly assigned to breakout groups led by the international experts (Predrag Kukic, Jean-Lou Dorne, Iris Mangas, Andrea Terron) and were invited to discuss and propose potential Swiss case studies with the experts that are suitable for follow-up through NAM-based assessment. The case study example pool was provided by representatives of the Swiss Authorities before the in-person workshop. The group discussions circled around key subjects of case study generation, including problem formulation, relevant compounds and disease areas, assessment strategies, and the Swiss-specific regulatory context. Therefore, they layed the ground for prioritization of case studies for final selection.

7.3.2 WORKSHOP PARTICIPANTS

A total of 28 participants attended the workshop with a majority (19) from the Swiss authorities (9 from FOPH; 5 from FSVO; 3 from SECO; and 2 from Swissmedic), the 4 invited international experts (Predrag Kukic, Jean-Lou Dorne, Iris Mangas, Andrea Terron) and 5 SCAHT directorate members (Figure 10).

Figure 10. Number and stakeholder distribution of workshop participants attending the SCAHT in-person workshop in Basel on November 11th 2024.

7.3.3 WORKSHOP OUTCOME

The workshop successfully initiated a cross-agency dialogue on how Switzerland can contribute concrete examples to the global advancement of modern, science-based regulatory practices. The pre-defined 8 potential case studies were discussed with the international experts and identified as a valuable pool for selection.

Table 13 collects the case studies and summarizes their respective discussion points.

Table 13: Summary of collected Swiss case studies proposed by the different Swiss Agencies.

Number (Agency)	CASE STUDY	Discussion points
1 (BLV)	Exposure to Succinate Dehydrogenase Inhibitors (SDHIs) leading to cancer	Question: Do SDHI fungicides produce the Adverse Outcome (AO) `Uterine Adenocarcinoma`? Regulatory Context: CLP and Pesticide risk assessment (RA). Data: data-poor, ANSES RA available. Not Swiss-specific Comments: Syngenta data not publicly available, difficult to publish.
2 (FOPH)	Understand the effects/potency of the BPA substitute BPTMC	Question: Understand hazard and relative potency factor of BPTMC compared to other BP. Regulatory Context: CLP; Swiss regulatory dossier & in silico prediction available Data: data-poor Swiss-specific due to Swiss production/export Comments: RA very work-intensive and a very limited amount of in vivo data available
3 (FOPH)	Assessment of the DNT effects of metal mixtures	Question: Understand the metal mixture (Hg, Cd, As, Pb, (U)) hazard/risk for DNT. Regulatory Context: REACH aggregate exposure RA Data: data-rich Can be assessed in a Swiss-specific way due to availability of environmental monitoring data Comments: Due to data-richness relatively easy, might require mixture testing in the DNT IVB for confirmation.
4 (FOPH)	Fabric softeners and skin sensitization	Question: Do fabric softeners/their mixtures with perfume cause skin/respiratory sensitization? Regulatory Context: CLP, REACH aggregate exposure RA Data: data-poor Not Swiss-specific

Number (Agency)	CASE STUDY	Discussion points
		Comments: DA on skin sensitization available, OECD guideline No 497.
5 (FOPH)	Can we explain the region-specific overrepresentation of Thelarche in Switzerland?	Question: Can we substantiate the geographic localization of thelarche in Switzerland? Is there epidemiological evidence of chemical exposure association with thelarche? Regulatory Context: - Data: data-poor (6 cases in the Swiss Jura) Swiss-specific Comments: Very difficult due to much restricted data.
6 (FOPH)	Respiratory Sensitization	Question: CH specific request for testing lung sensitization (no legal data requirement) Regulatory Context: no current regulatory require Data: - Swiss-specific Comments: Currently no appropriate in vitro models. There is the need to explore potentially relevant NAMs for prediction assessment. Potential NAMs for performance for volatile mixtures should be assessed.
7 (BLV)	Nanocellulose as a food preservative	Question: Is there a human health risk when using nanocellulose as a food preservative for cucumbers (workers, consumers)? Regulatory Context: no current regulatory require; food safety Data: - Swiss-specific Comments: context assessment (population at risk), exposure assessment, fate description, hazard identification and characterization; IATA framework with WoE approach very time-consuming.
8 (SCAHT/ MAK comm.)	1,2-dichloropropane as a human carcinogen	Question: Can we assess the human carcinogenic potential for the gall ducts of 1,2-dichloropropane using NAMs? Regulatory Context: CLP Data: data-rich Not Swiss-specific Comments: Very interesting case study because the animal experiments do not predict the human carcinogenic potential of 1,2-dichloropropane.

From these proposed case studies two were prioritized for future elaboration. Criteria for prioritization were:

- SCAHT expertise.
- Relevance for Switzerland.
- Opposing cases for data-rich and data-poor.
- Opinions from international experts.
- Results from the discussions in the breakout groups.
- Capacity and capability considerations: Feasibility and resource demand: Consideration of available data, expertise, infrastructure, and cost implications.
- Mechanistic interpretability: Ability to link results to Adverse Outcome Pathways (AOPs) or other mechanistic frameworks.
- Alignment with international initiatives: Synergy with ongoing OECD, EFSA, or ECHA efforts to facilitate harmonization and mutual acceptance.
- Stakeholder engagement and demonstration value: Potential to stimulate dialogue among regulators, academia, and industry, and to showcase innovative applications of NAMs in chemical safety assessment.

Together, these criteria aim to ensure that the selected case studies are scientifically credible, policy-relevant, and representative of the challenges and opportunities associated with implementing NAMs in regulatory toxicology.

Therefore, as a result of the prioritization exercise, the following case studies were chosen for further elaboration:

Number (Agency)	CASE STUDY
2 (FOPH)	Understand the effects/potency of the BPA substitute BPTMC
3 (FOPH)	Assessment of the DNT effects of metal mixtures

In the next chapter (Technical Report D4) preliminary data on these case studies will be presented. In addition, two strategies and two action plans were elaborated that indicate the next steps and actions on these case studies in the future. That two different strategies had to be worked out for D4 indicates that the case studies were well chosen and that depending on the regulatory question and data availability, different actions have to be taken for their elaboration (Leist et al., 2025).

8 TECHNICAL REPORT D4: SUMMARY OF THE SCENARIO-BASED CASE STUDIES (INITIATION)

Case studies were identified as key technical and regulatory needs for the future of NAMs in regulatory risk assessment. To support the uptake of NAMs in regulatory frameworks and enhance Switzerland`s visibility in the International NGRA community, the FOPH with other Swiss agencies and the SCAHT decided on two Swiss case studies, which will be elaborated in the future. Therefore, regulatory problems were formulated that will be addressed with available NAM data. The two cases are:

1. Understanding the effects/potency of the BPA substitute BPTMC
2. Assessment of the DNT effects of metal mixtures

As the two cases do not have the same data availability (data poor vs. data rich), strategies differ between both.

8.1 CASE STUDY 1: UNDERSTANDING THE EFFECTS/POTENCY OF THE BPA SUBSTITUTE BPTMC

8.1.1 INTRODUCTION

BPA is a chemical substance (CAS number 80-05-7 (US EPA, 2024a)) produced in large scales, with broad industrial application. Since 1950 it has been employed in the production of polycarbonate plastics and epoxy resins, materials used in drink water bottles coating, kitchenware, and canned food and beverages. The non-food applications of BPA involve thermal printing papers, toys, and medical devices. It is known that BPA leaches from packaging and storage containers, contaminating food and beverages, thus, offering risk to human health. Recently, BPA utilization has been gradually restricted in the European Union (EU) due to its harmful effects on immune, reproductive, and endocrine systems. In 2010, BPA-based polycarbonate uses in baby bottles was banned in France. In the following year, the banishment was also adopted by the EU. In 2015, BPA utilization in food contact material was prohibited in France and one year later, the ECHA added BPA to the list of “very high concern” chemicals (ChemTrust, 2025). In parallel, European authorities discouraged the

utilization of the BPA alternative BPS due to a toxicological profile similar to BPA. In 2020, thermal paper containing BPA was banned in Europe. Considering the toxicological concerns associated with BPS and the availability of bisphenol-free thermal paper, Switzerland implemented a ban on thermal paper containing ≥0.02% by mass of BPA or BPS, effective from 15 December 2020 (Demierre et al., 2024). At the end of 2024, the EU commission prohibited the utilization of BPA and other bisphenols in food contact material, providing an 18-moth phase out period (European Commission, 2024).

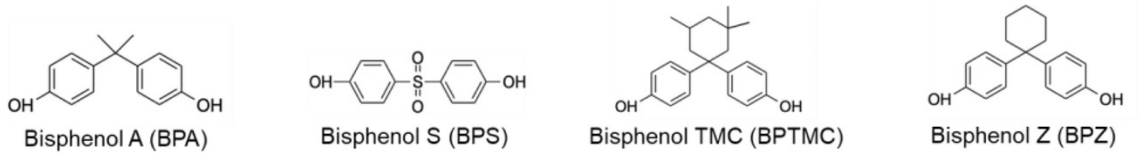


Figure 11. BPA and its analogues BPS, BPZ, and BPTMC. Adapted from Lucarini et al., 2023.

Facing the unavoidable discontinuation of BPA, alternative bisphenol compounds are constantly being developed, such as such as BPZ and BPTMC (Figure 11). BPA alternative compounds often share structural similarities to their parental molecule, raising concerns on their safety. However, toxicological data of most of the new BPA analogues are extremely scarce.

One such compound, BPTMC (CAS number 129188-99-4) is currently preregistered under the REACH framework (ECHA, 2021b). The incomplete ECHA dossier suggests that the compound may be exempt from registration, not manufactured or imported within the EU, or produced/imported below the one-ton-per-year threshold. Although BPTMC is registered in Switzerland, it is unclear if it is produced or imported into the country. Interestingly, a recent study found no detectable levels of BPTMC in food samples from Swiss and EU markets (Lucarini et al., 2023).

8.1.2 METHODOLOGY

To develop the BPTMC case study, we will apply a multi-tiered risk assessment approach as suggested by the Alternative Safety Profiling Algorithm (ASPA) graphically described in Figure 12 (Leist et al., 2025). This workflow has been developed within the collaborative EU project

RiskHunt3R[1]of the ASPIS cluster[2]. ASPA provides a broad purpose, transparent, and reproducible risk assessment workflow that enables the documentation and integration of all information types relevant to NGRA. Its goal is to make safety assessments fully traceable for end users (e.g., regulators) by clearly outlining the steps and decisions that led to the final outcome and the rationale behind them.

Each case starts with the problem formulation and is followed by a collection of data. Depending on data availability, different paths are taken.

The Threshold of Toxicological Concern (TTC) approach has been developed to qualitatively assess the risk of low-level substances in the diet. It can be used for an initial assessment of a substance to determine whether a comprehensive risk assessment is required. It is an important science-based approach for prioritizing assessment of those chemicals with low-level exposures that require more data over those that can be presumed to present no appreciable human health risk. (EFSA[3]). In case the TTC is applicable, a workflow exit/report follows.

In case a TTC is not applicable, it is questioned if a PoD and a human equivalent dose (HED[4]) using an available PBK model can be derived. In case information is not sufficient, a read-across module is foreseen as the next step.

[1] https://www.risk-hunt3r.eu

[2] https://aspis-cluster.eu

[3] https://www.efsa.europa.eu/en/topics/topic/threshold-toxicological-concern

[4] Note that various alternative terms for HED are in use in various contexts and regulations. Examples are reference points (RP) by EFSA, reference doses (RfD) or in vivo point-of-departure (PoD) as well as equivalent administered dose (EAD) or administered equivalent dose (AED) as used by the US EPA.

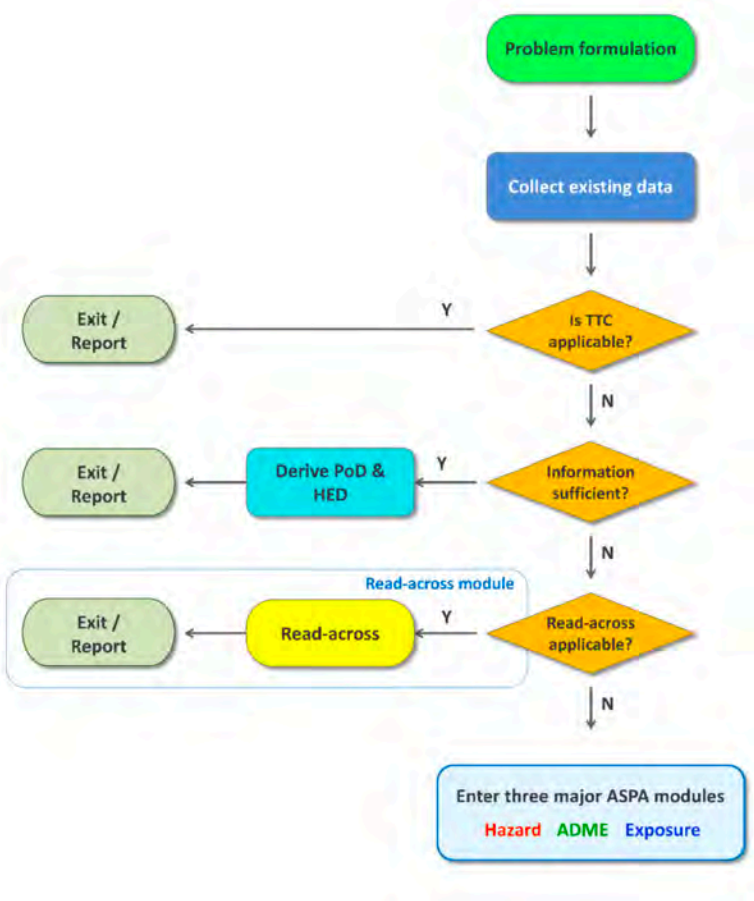


Figure 12. General principles of the first steps in the ASPA workflow (Leist et al., 2025). Similar assessment will be applied for the BPTMC case study. TTC – Threshold of Toxicological concern; PoD – Point of Departure; HED – Human Equivalent Dose; ADME – Absorption, Distribution, Metabolism, Excretion.

8.1.3 BPTMC CASE STUDY WORKFLOW WITH PRELIMINARY RESULTS

A. Problem formulation

Understand the hazard potential including mode of action (MoA) of BPTMC and compare it to BPA.

B. Collecting existing data

We collected existing scientific data on BPTMC by searching across biomedical and life sciences literature archives, open-access chemical databases, and web-based platforms dedicated to sharing mechanistic and AOP-related information.

- **BPTMC scientific publications**

A literature screening conducted in PubMed and Web of Science identified 20 publications on BPTMC available up to October 2025. This number of available studies on the toxicity profile of BPTMC is very limited. We analysed both review and research articles on BPTMC, covering a diverse range of topics such as plant uptake, analytical method development, food contact materials, human and environmental biomonitoring, as well as developmental, reproductive, and endocrine toxicity studies (Table 14).

Table 14: List of publications mentioning BPTMC identified through PubMed and Web of Science searches.

Title	Topic	Reference
Bisphenol TMC disturbs mitochondrial activity and biogenesis, reducing lifespan and healthspan in the nematode Caenorhabditis elegans	Developmental and reproductive toxicity	(Rathor et al., 2025)
Development of a sensitive UPLC-MS/MS method for simultaneous determination of 15 Bisphenol A analogues in human serum and urine: Application of paired samples and global exposure assessment	Analytical method, Human biomonitoring	(Hu et al., 2025)
The Effects of Bisphenol A and its Analogs on Steroidogenesis in MA-10 Leydig Cells and KGN Granulosa Cells	Endocrine disruption	(Iskandarani et al., 2025)
Adverse outcomes of the newly emerging bisphenol A substitutes	Review article	(Franko et al., 2024)
A prioritization strategy for functional alternatives to bisphenol A in food contact materials	Review article	(van den Brand et al., 2024)

Developmental effects and lipid disturbances of zebrafish embryos exposed to three newly recognized bisphenol A analogues	Developmental toxicity	(Zhao et al., 2024)
Congener-specific uptake and accumulation of bisphenols in edible plants: Binding to prediction of bioaccumulation by attention mechanism multi-layer perceptron machine learning model	In silico plant uptake	(Yang et al., 2023)
Gas chromatography–mass spectrometric determination of bisphenol residues by dispersive SPE followed by activated-carbon spheres cleanup from fish feed samples	Analytical method	(Talari et al., 2023)
Reproductive toxicity of emerging plasticizers, flame retardants, and bisphenols, using culture of the rat fetal testis	Reproductive toxicity	(Tardif et al., 2023)
Simultaneous Quantification of 16 Bisphenol Analogues in Food Matrices	Food contact material	(Lucarini et al., 2023)
Toxicity and Estrogenicity of Bisphenol TMC in Oryzias melastigma via In Vivo and In Silico Studies	Endocrine disruption & developmental toxicity	(Li et al., 2023)
Twenty bisphenol analogues in take-out polystyrene-made food containers: concentration levels, simulated migration, and risk evaluation	Food contact material	(Zhao et al., 2023)

High-content imaging analyses of the effects of bisphenols and organophosphate esters on TM4 mouse Sertoli cells	Reproductive toxicity	(Rajkumar et al., 2022)
Bisphenols emerging in Norwegian and Czech aquatic environments show transthyretin binding potency and other less-studied endocrine-disrupting activities	Endocrine disruption	(Šauer et al., 2021)
Elucidation of the Effects of Bisphenol A and Structural Analogs on Germ and Steroidogenic Cells Using Single Cell High-Content Imaging	Endocrine disruption	(Rajkumar et al., 2021)
Investigation of the migration of bisphenols from baby bottles and sippy cups	Food contact material	(Siddique et al., 2021)
Exposure to New Emerging Bisphenols Among Young Children in Switzerland	Human biomonitoring	(Lucarini et al., 2020)
Occurrence, mass loads and risks of bisphenol analogues in the Pearl River Delta region, South China: Urban rainfall runoff as a potential source for receiving rivers	Environmental biomonitoring	(Huang et al., 2020)
BPA, BADGE and analogues: A new multi-analyte LC-ESI-MS/MS method for their determination and their in vitro (anti)estrogenic and (anti)androgenic properties	Analytical method	(van Leeuwen et al., 2019)
Selected bisphenols and phthalates screened for estrogen and androgen disruption by in silico and in vitro methods	Endocrine disruption	(Dvorakova et al., 2018)

The BPTMC key findings described in research papers, including two Swiss studies, are summarized below.

Bisphenol TMC disturbs mitochondrial activity and biogenesis, reducing lifespan and healthspan in the nematode *Caenorhabditis elegans* (Rathor et al., 2025): In *C. elegans*, 1 mM BPTMC disrupted mitochondrial function and biogenesis. These effects were accompanied by developmental delays, reduced reproduction, diminished longevity, reduced motility, and decreased stress resistance, indicating that BPTMC compromises both lifespan and health span.

Development of a sensitive UPLC-MS/MS method for simultaneous determination of 15 Bisphenol A analogues in human serum and urine: Application of paired samples and global exposure assessment (Hu et al., 2025). A highly sensitive ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method capable of simultaneously detecting 15 bisphenol A analogues, including BPTMC, in human serum and urine samples was developed and validated. Forty-four paired urine and serum samples from children aged 7–10 years living in the Huai River Basin (China) were analysed for bisphenol biomonitoring. BPTMC was not detected in any urine samples, but it was detected in 13.6% of serum samples (LOD = 0.012 µg/L), with mean and maximum concentrations of 0.014 µg/L and 0.207 µg/L, respectively. Although the number of positive samples was limited, these findings indicate that serum is a more appropriate matrix for BPTMC biomonitoring, an important consideration for assessing BPTMC exposure and human health risks associated with this compound.

The Effects of Bisphenol A and its Analogs on Steroidogenesis in MA-10 Leydig Cells and KGN Granulosa Cells (Iskandarani et al., 2025). The effects of 0.01-50 µM BPTMC on steroid hormone synthesis in MA-10 Leydig and KGN granulosa cells was investigated. BPTMC exposure altered steroidogenic pathways, evidenced by disrupted oestradiol and progesterone production. The results demonstrated that BPTMC acts as an endocrine disruptor, capable of interfering with steroid hormone production, similar to BPA.

Developmental effects and lipid disturbances of zebrafish embryos exposed to three newly recognized bisphenol A analogues (Zhao et al., 2024). The developmental effects of 0.1–1000 µg/L BPTMC was assessed in zebrafish embryos. BPTMC exposure caused developmental disorders, inhibition of yolk sac absorption, altered heart rate, teratogenic effects, alongside increased lipid accumulation in the yolk sac. These findings indicate that BPTMC disrupts both embryonic development and lipid metabolic processes in aquatic organisms.

Congener-specific uptake and accumulation of bisphenols in edible plants: Binding to prediction of bioaccumulation by attention mechanism multi-layer perceptron machine learning model (Yang et al., 2023). Using a machine learning approach, a high potential for bioaccumulation in plant was predicted for BPTMC.

Gas chromatography-mass spectrometric determination of bisphenol residues by dispersive solid phase extraction followed by activated carbon spheres cleanup from fish feed samples (Talari et al., 2023). To analyse up to 11 bisphenols, including BPTMC, in commercial fish feed samples, a gas chromatography-MS method was developed. BPTMC was detected in several feed samples at trace concentrations, confirming its occurrence in aquaculture inputs and suggesting a potential route of exposure for aquatic organisms through contaminated feed.

Reproductive toxicity of emerging plasticizers, flame retardants, and bisphenols, using culture of the rat fetal testis (Tardif et al., 2023). The reproductive effects of 10 µM BPTMC was assessed using an ex vivo culture model of rat foetal testes. BPTMC increased testosterone production, and luteinizing hormone-stimulated testosterone secretion, and Leydig cell density and surface area. These findings indicate that BPTMC can modulate steroidogenic activity in the developing testis, suggesting androgenic potential and possible disruption of foetal testicular development.

Simultaneous Quantification of 16 Bisphenol Analogues in Food Matrices (Lucarini et al., 2023). A liquid chromatography tandem mass spectrometry method was established and validated for the simultaneous quantification of 16 bisphenol analogues, including BPTMC, in various food matrices.

Toxicity and Estrogenicity of Bisphenol TMC in *Oryzias melastigma* via In Vivo and In Silico Studies (Li et al., 2023). The developmental toxicity and estrogenic potential of BPTMC was assessed in *Oryzias melastigma*. Embryos exposed to 0.25–2000 µg/L BPTMC exhibited altered hatching, malformation, heart rate, swimming activity, inflammatory response, and the expression of oestrogen-responsive markers consistent with endocrine disruption. Molecular docking supported these findings, showing strong binding affinity of BPTMC to oestrogen receptors. Overall, BPTMC demonstrated dose-dependent developmental toxicity and estrogenic activity in fish embryos.

Twenty bisphenol analogues in take-out polystyrene-made food containers: concentration levels, simulated migration, and risk evaluation (Zhao et al., 2023). The presence and migration behaviour of BPTMC in polystyrene-made plastic products was assessed using high-performance liquid chromatography tandem mass spectrometry. BPTMC was detected in several container samples, showing potential to migrate into food.

High-content imaging analyses of the effects of bisphenols and organophosphate esters on TM4 mouse Sertoli cells (Rajkumar et al., 2022). TM4 mouse Sertoli cells were exposed to concentrations ranging from 0.001 to 100 µM BPTMC, which exhibited the highest cytotoxic potency among tested bisphenols. It significantly increased lipid droplet formation and lysosomal alterations while reducing cell viability, suggesting potential reproductive toxicity caused by BPTMC.

Bisphenols emerging in Norwegian and Czech aquatic environments show transthyretin binding potency and other less-studied endocrine-disrupting activities (Šauer et al., 2021). BPTMC was found in sediments and surface waters. In in vitro transthyretin binding assays, BPTMC demonstrated anti-progestogenic and anti-androgenic activity, indicating that BPTMC exhibits notable endocrine-disrupting activity and environmental persistence.

Elucidation of the Effects of Bisphenol A and Structural Analogs on Germ and Steroidogenic Cells Using Single-Cell High-Content Imaging (Rajkumar et al., 2021). Using high-content imaging, up to 100 µM BPTMC was tested on germ and steroidogenic (MA-10 Leydig and KGN granulosa) cell lines. BPTMC exhibited among the strongest cytotoxic effects, besides altering

lipid droplets formation in the tested cells. Overall, BPTMC was the most potent bisphenol studied.

Investigation of the migration of bisphenols from baby bottles and sippy cups (Siddique et al., 2021). BPTMC presence in from baby bottles and sippy cups was analysed using liquid chromatography mass spectrometry. BPTMC was detected and shown to migrate into food simulants, particularly at elevated temperatures. Although migration levels were generally low, the findings demonstrated that BPTMC can leach from consumer products intended for infants, raising concerns about potential early-life exposure to this emerging bisphenol analogue.

Exposure to New Emerging Bisphenols Among Young Children in Switzerland (Lucarini et al., 2020). A human biomonitoring study to assess exposure to bisphenols among young children in Switzerland was performed by applying a validated LC–MS/MS method, analysing infants and toddlers urine from diapers. The concentrations of BPTMC were below the limit of quantification.

Occurrence, mass loads and risks of bisphenol analogues in the Pearl River Delta region, South China: Urban rainfall runoff as a potential source for receiving rivers (Huang et al., 2020). Environmental occurrence and distribution of bisphenol analogues in the Pearl River Delta region of South China were measured HPLC–MS/MS. BPTMC was detected in urban rainfall runoff and receiving river waters, indicating widespread environmental presence.

BPA, BADGE and analogues: A new multi-analyte LC-ESI-MS/MS method for their determination and their in vitro (anti)estrogenic and (anti)androgenic properties (van Leeuwen et al., 2019). A new multi-analyte LC-ESI-MS/MS method to quantify 17 bisphenol analogues, including BPTMC, in food, beverages and drinkware was developed. The endocrine activity of BPTMC was assessed in Yeast based bioassays, and BPTMC displayed higher anti-androgenic potency than BPA.

Selected bisphenols and phthalates screened for estrogen and androgen disruption by in silico and in vitro methods (Dvořáková et al., 2018). The endocrine-disrupting potential of

selected bisphenols, including bisphenol BPTMC using combined in silico molecular docking and in vitro receptor-based assays. Strong binding affinity of BPTMC to oestrogen (agonist) and androgen (antagonist) receptors.

Collectively, the available evidence indicates that BPTMC exhibits biological activity and environmental behaviour consistent with an emerging endocrine disrupting compound. In parallel, analytical and environmental studies confirm its occurrence in consumer products and aquatic environments, pointing to widespread but insufficiently characterized exposure. These findings highlight the need for comprehensive toxicological, mechanistic and exposure assessments to better define the human health and environmental risks associated with BPTMC.

Following FOPH request, 12 additional papers covering, food contact materials, environmental biomonitoring, adipogenesis, as well as endocrine toxicity studies, were incorporated in this report (Table 15).

Table 15: List of publications mentioning BPTMC identified by FOPH reviewers.

Title	Topic	Reference
Occurrence, ecological risk and estrogenic effect of 19 bisphenol analogues in the surface water used for drinking water in Shanghai, China	Occurrence, ecological risks, and predicted estrogenic effects	(Wang et al., 2025)
The obesogenic effects of Bisphenol A and its analogues are differentially regulated via PPARγ transactivation in mouse 3T3-L1 cells	Adipogenesis	(Crosthwait et al., 2025)
Concentrations, composition profiles, and in vitro-in silico-based mixture risk assessment	Human dietary exposure: No results for BPTMC	(Chiu et al., 2025)

of bisphenol A and its analogs in plant-based foods		
Exposure to endocrine disrupting chemicals from beverage packaging materials and risk assessment for consumers.	Food contact material	(Marchiandi et al., 2024)
Interaction of Bisphenol A and Its Analogs with Estrogen and Androgen Receptor from Atlantic Cod (Gadus morhua).	Endocrine disruption	(Goksøy et al., 2024)
In silico profiling of endocrine-disrupting potential of bisphenol analogues and their halogenated transformation products.	Endocrine disruption	(Nowak & Jakopin, 2023)
Simultaneous measurement of 16 bisphenol A analogues in house dust and evaluation of two sampling techniques	Analytical method	(Fan et al., 2021)
A tiered high-throughput screening approach for evaluation of estrogen and androgen receptor modulation by environmentally relevant bisphenol A substitutes	Endocrine disruption	(Keminer et al., 2020)
Mechanistic in silico modeling of bisphenols to predict estrogen and glucocorticoid disrupting potentials.	Endocrine disruption	(Chen et al., 2020)

Molecular Initiating Events of Bisphenols on Androgen Receptor-Mediated Pathways Provide Guidelines for in Silico Screening and Design of Substitute Compounds	Endocrine disruption	(Chen et al., 2019)
In silico molecular interaction of bisphenol analogues with human nuclear receptors reveals their stronger affinity vs. classical bisphenol A	Endocrine disruption, molecular docking	(Sharma et al., 2018)
Molecular interactions of bisphenols and analogs with glucocorticoid biosynthetic pathway enzymes: an in silico approach	Endocrine disruption, molecular docking	(Verma et al., 2017)

The BPTMC key findings described in research papers are summarized below.

Occurrence, ecological risk and estrogenic effect of 19 bisphenol analogues in the surface water used for drinking water in Shanghai, China (Want et al., 2025). BPTMC was detected as one of the bisphenol analogues present in the surface water and drinking water sources, being found at relatively low concentrations. Although BPTMC does not appear to pose a significant standalone risk based on concentration its risk quotient was or RQ values, it may still contribute to the overall endocrine-disrupting activity arising from multiple bisphenols co-occurring in the aquatic environment.

The obesogenic effects of Bisphenol A and its analogues are differentially regulated via PPARγ transactivation in mouse 3T3-L1 cells (Crosthwait et al., 2025). The effects of fifteen BPA analogues on adipogenesis in the mouse 3T3-L1 cells were tested to determine their adipogenic activity relative to BPA. BPTMC has no effect on the levels of adipogenic genes *Fabp4, Plin1*, *Lpl.*

Concentrations, composition profiles, and in vitro-in silico-based mixture risk assessment of bisphenol A and its analogs in plant-based foods (Chiu et al., 2025). The paper investigates the occurrence of bisphenol analogues in plant-based foods in Taiwan, estimates human dietary exposure across age groups, and evaluates mixture risks aligned with oestrogen receptor activity. Notably, the study does not report any findings related to BPTMC.

Exposure to endocrine disrupting chemicals from beverage packaging materials and risk assessment for consumers (Marchiandi et al., 2024). The study evaluated the occurrence of endocrine-disrupting chemicals in beverages packaged in various materials (e.g., plastic, glass, carton, aluminium, and cans) available to consumers in a major Australian metropolitan region. BPTMC was not detected in any of the tested samples.

Interaction of Bisphenol A and Its Analogs with Estrogen and Androgen Receptor from Atlantic Cod (Gadus morhua) (Goksøyet al., 2024). The study aimed to evaluate whether 11 BPA analogues disrupt oestrogen or androgen receptor signalling pathways in Atlantic cod. Overall, BPTMC displayed mixed estrogenic and antiandrogenic effects despite relatively low potency. It acted as a weak oestrogen receptor agonist, antagonized oestrogen signalling, and inhibited androgen receptor activity.

In silico profiling of endocrine-disrupting potential of bisphenol analogues and their halogenated transformation products (Nowak & Jakopin, 2023). This in silico study evaluated the endocrine-disrupting potential of bisphenol analogues and their halogenated derivatives. BPTMC was identified as one of the bisphenols with the highest predicted endocrine-disrupting potential, showing strong binding to multiple nuclear receptors, particularly ERα/ERβ (agonist and antagonist), androgen receptor (antagonist), mineralocorticoid receptor, and thyroid receptor β.

Simultaneous measurement of 16 bisphenol A analogues in house dust and evaluation of two sampling techniques (Fan et al., 2021). A sensitive GC-MS/MS method to measure 16 bisphenol analogues in indoor dust and applied it to Canadian household samples. BPTMC was detected in 47% of the samples with a maximum of 0.309 µg/g.

A tiered high-throughput screening approach for evaluation of estrogen and androgen receptor modulation by environmentally relevant bisphenol A substitutes (Keminer et al., 2020). The endocrine potential of BPA analogues used in environmentally relevant applications by using a biochemical and cell-based assays. BPTMC bound androgen and oestrogen receptors but showed weak co-activator recruitment and receptor activation in reporter assays.

Mechanistic in silico modeling of bisphenols to predict estrogen and glucocorticoid disrupting potentials (Chen et al., 2020). In this study, cell-based reporter assays with mechanistic computer modelling to link receptor conformations to agonist, antagonist, or mixed effects were used to assess how bisphenols disrupt Erα and glucocorticoid receptor signalling. BPTMC acted as a pure Erα antagonist and as a pure glucocorticoid receptor agonist.

Molecular Initiating Events of Bisphenols on Androgen Receptor-Mediated Pathways Provide Guidelines for in Silico Screening and Design of Substitute Compounds (Chen et al., 2019). The study investigated how bisphenols disrupt androgen receptor signalling to inform safer substitute design, using cell-based assays and molecular dynamics to assess receptor binding, coregulator recruitment, and antagonist activity. BPTMC showed strong androgen receptor binding and anti-androgenic potential.

In silico molecular interaction of bisphenol analogues with human nuclear receptors reveals their stronger affinity vs. classical bisphenol A (Sharma et al., 2018). The study aimed to assess bisphenol analogues endocrine-disrupting potential and interaction with human peroxisome proliferator-activated receptors (PPARs) and retinoid X receptors (RXRs). Binding efficiency was evaluated through molecular docking. BPTMC showed interaction with PPARs and RXRs.

Molecular interactions of bisphenols and analogs with glucocorticoid biosynthetic pathway enzymes: an in silico approach (Verma et al., 2017). The study aimed to evaluate whether various bisphenols can interfere with enzymes involved in glucocorticoid synthesis, by

applying computer-based molecular docking. BPTMC showed moderate binding affinity to several enzymes of the glucocorticoid synthesis pathway.

- **BPTMC experimental and predicted toxicity**

In addition to PubMed and Web of Sciences, we search for unpublished BPTMC data using on the platform CompTox Chemicals Dashboard, developed by the U.S. Environmental Protection Agency (US EPA), which is an open-access platform that integrates chemical, toxicological, and exposure data from multiple sources (US EPA, 2024d). It integrates data from ToxCast, PubChem, PubChem BioAssay, among others. The data from PubChem BioAssay showed that cell-based receptor binding and reporter gene assays available have identified BPTMC as an agonist of human oestrogen receptors α (ERα) and β (ERβ), indicating its potential to activate oestrogen-responsive signalling pathways (PubChem, 2023; US EPA, 2024b). This oestrogenic activity suggests that BPTMC may mimic endogenous hormones, contributing to endocrine-disrupting effects similar to those observed for BPA.

In addition to BPTMC PubChem bioactivity data, comparative toxicity profiles of BPTMC, BPZ, and BPA, performed by the platform CompTox, were collected (Figure 13). This application predicts toxicity type (human and environmental health) and environmental fate based on structural similarity. Based on expert judgment (Jean-Lou Dorne, EFSA) during the workshop (D3, this report), BPZ was identified as a suitable reference compound for BPTMC. Unlikely BPZ and in contrast to previously described in vitro data, the in silico toxicity prediction for BPTMC did not indicate endocrine, reproductive, or developmental toxicity (US-EPA, 2024a). Although BPTMC acute toxicity was not predicted by CompTox, the UN GHS classification of this compound includes hazard alerts for skin, eye, and respiratory irritation (US-EPA, 2024c). This discrepancy between predicted, in vitro, and classified hazards highlights the need for additional studies to clarify the toxicological profile of BPTMC.

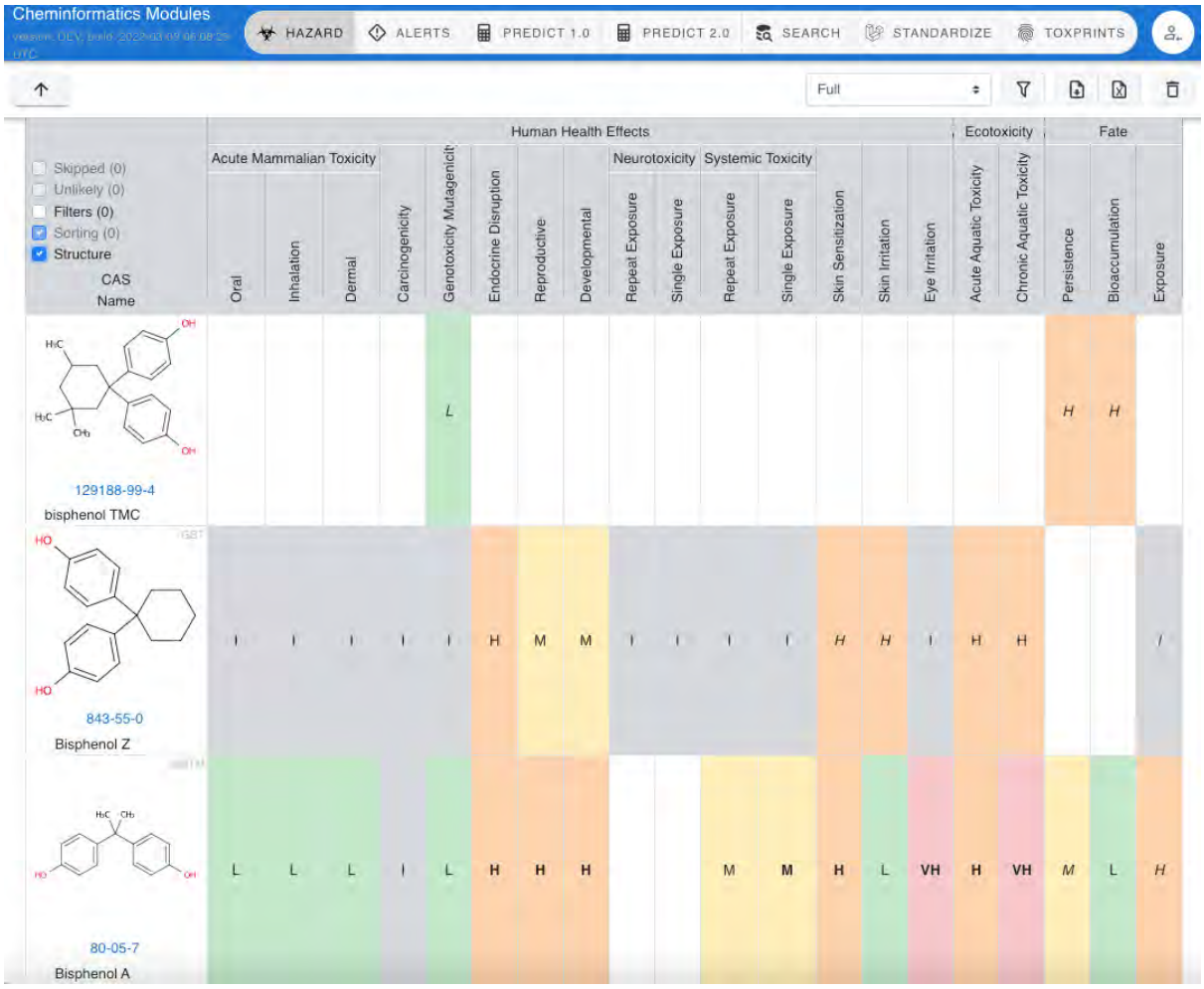


Figure 13. Human health effects, ecotoxicity, and environmental fate of BPTMC, BPZ, and BPA, generated by CompTox (US EPA, 2024a). Toxicity: VH – Very High; H – High; M – Medium; L – Low; I – Inconclusive.

Consistent with the UN GHS hazard statement “H410 (100%): Very toxic to aquatic life with long-lasting effects,” in silico assessments also highlighted a high potential for persistence and bioaccumulation, raising concerns about its long-term environmental impact (US-EPA, 2024c, 2024d).

To complement the CompTox bioactivity results, we also considered predictions from computational in silico screening tools for endocrine disruption (CIST-ED), as suggested by FOPH. CIST-ED integrates QSARs, molecular docking, and receptor-binding prediction models covering key endocrine pathways (Mani et al., 2025). For data-poor bisphenols such as BPTMC, these in silico tools help identify potential receptor interactions that may not yet be

captured in CompTox assays, thereby strengthening the mechanistic interpretation and ensuring a more balanced assessment of endocrine-disrupting potential.

Within the SCAHT research network, the MolSysTox laboratory led by Prof. Alex Odermatt is currently conducting in vitro studies to assess the effects of BPTMC. ERα activation by BPTMC has been assessed both in transfected HEK cells and in MCF-7 cells, the latter of which endogenously express high levels of ERα. These studies are expected to be completed in 2025 and will provide essential data to support our BPTMC case study. The recently launched structured collection of tools of PARC, the Safe and Sustainable by Design (SSbD) Toolbox, can potentially be applied in the future to gather information on BPTMC toxicity.

- **Adverse outcome pathways (AOPs) concerning BPA MoA**

To complement the literature and database searches, the AOP-Wiki was explored to provide a mechanistic context for the potential biological effects of BPTMC (AOP-Wiki, 2025). By mapping these mechanistic pathways, the AOP-Wiki facilitates the identification of key events shared across chemicals with similar modes of action, supporting read-across approaches and the interpretation of in vitro and in silico data. This resource is particularly valuable for data-poor substances such as BPTMC, as it allows leveraging information from well-studied analogues like BPA to infer plausible mechanisms of toxicity and prioritize further testing.

Our screening in the AOP-Wiki identified four AOPs in which BPA is listed as a stressor. The resulting BPA AOP (network) is summarized in Figure 14.

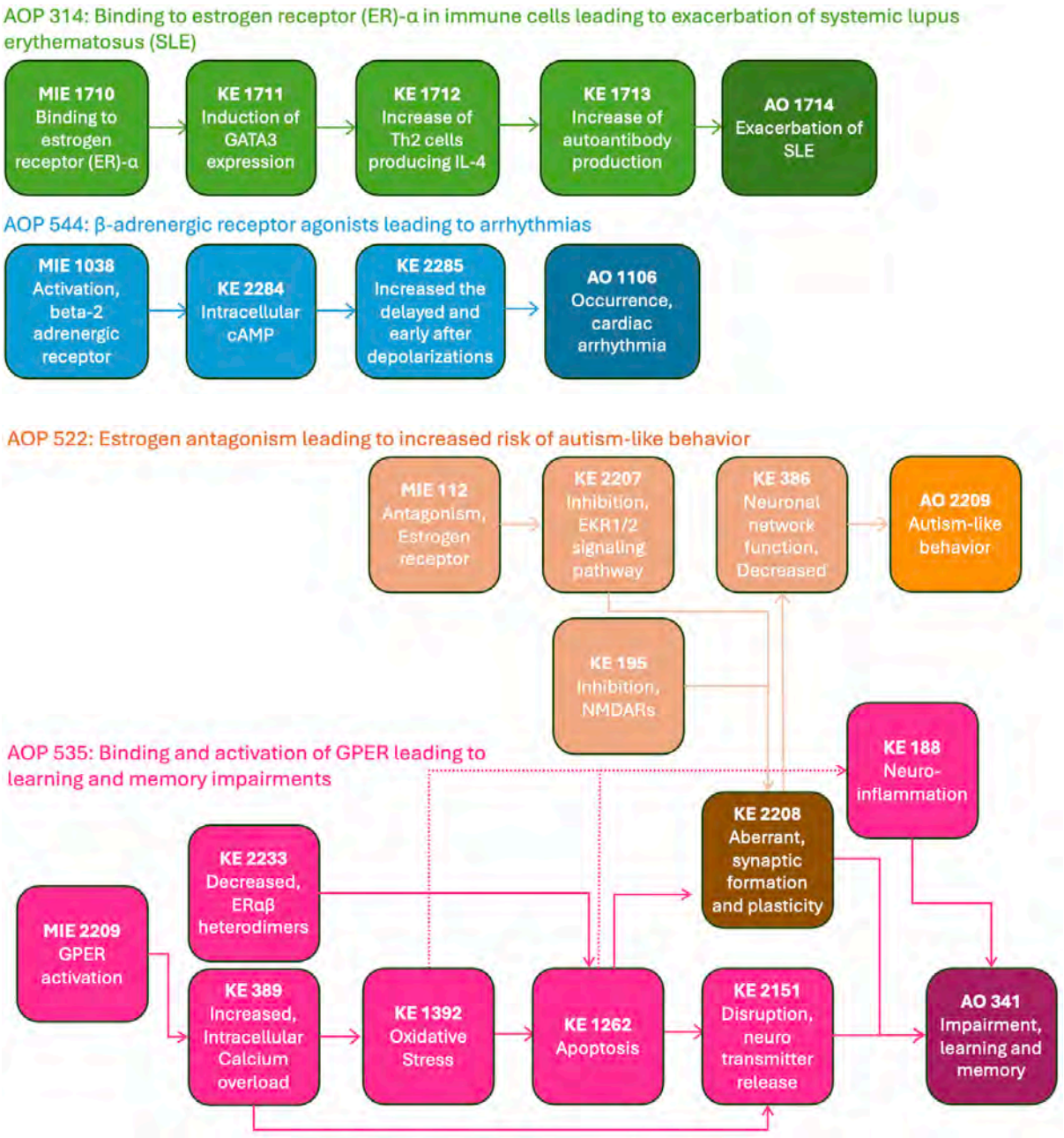


Figure 14. AOP networks among AOPs 314, 544, 522, and 535, which share BPA as prototypical stressors. MIE: Molecular Initiating Event. KE: Key Event. AO: Adverse Outcome. ER: oestrogen receptor. SLE: Systemic Lupus Erythematosus. cAMP: Cyclic adenosine monophosphate. NMDAR: N-methyl-D-aspartate receptor. GPER: G protein-coupled oestrogen receptor.

These AOPs are still under development and currently supported by moderate to weak evidence for the key event relationships. Therefore, further research is needed to strengthen and expand the mechanistic understanding of BPA-related pathways and, by extension, to elucidate the potential AOPs relevant to its analogues, such as BPTMC. Here, especially MIE-based BPTMC testing could inform on AOP relevance of chemical analogues.

- **Threshold of toxicological concern, Point of Departure, Human equivalent Dose**

Due to the limited data availability of BPTMC, we are currently unable to define a TTC, derive a PoD or HED. Therefore, according to the ASPA workflow (Leist et al., 2025), the next foreseen step is the performance of read-across.

- **Read-Across**

Read-across of BPTMC can be performed to predict its toxicological profile based on its structural and mechanistic similarity to other, well-characterized bisphenols. Like indicated above, BPZ was identified as a suitable reference compound based on expert judgment (Jean-Lou Dorne, EFSA). Our literature search retrieved 95 BPZ-related publications in PubMed, which need further analyses in detail in the future. Moreover, BPZ is among the seven bisphenol alternatives prioritized by WP5 of PARC due to identified data gaps (Holden et al., 2023), and the data generated by WP5 might be use to endorse our BPTMC read-across.

Importantly, the read-across assessment needs to be conducted in a transparent and systematic manner, with clear scientific justification and critical evaluation, in line with the EFSA Guidance on the use of read-across for chemical safety assessment in food and feed (Bennekou et al., 2025), justifying the selection of BPZ and potential other reference compounds.

8.1.4 SUMMARY, OUTLOOK AND CONCLUSION

- Our literature search identified limited data available for BPTMC. However, interaction with nuclear hormone receptors including ER, PPAR, GR and with the glucocorticoid biosynthetic pathways were identified. However, additional studies are required to fully characterize its hazard profile.
- The ongoing research on the oestrogenic activity of BPTMC conducted by the MolSysTox laboratory, led by Prof. Alex Odermatt, is expected to provide valuable insights into the endocrine-disrupting potential of BPTMC.
- Results from the Horizon Europe Project PARC (WP 5) might be leveraged to strengthen and contextualize the BPTMC case study.
- It is planned that this case study will be further developed under “WP3: Analysis of the adverse effects of plastic additives in relation to DART” (project: InPlastiMoA, contract 149000092), by expanding the AOP network and applying CIST-ED framework.

- The next steps of this case study are outlined in Figure 15 within a study work flow.

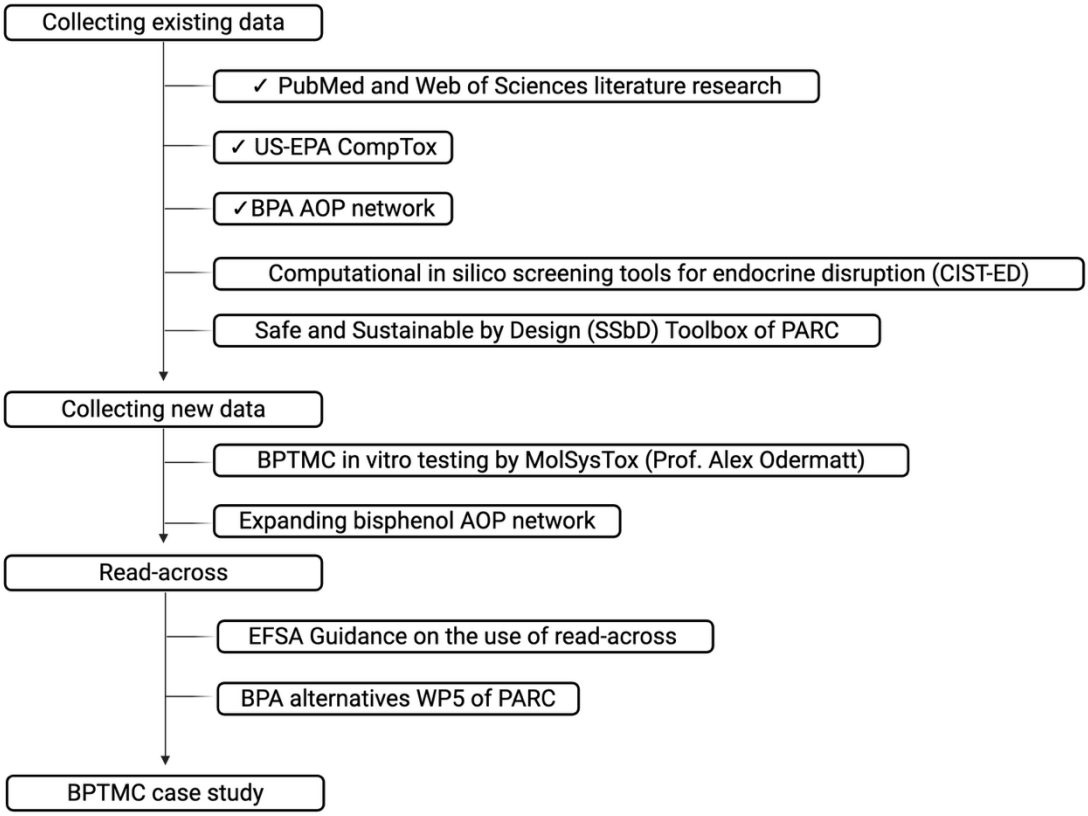


Figure 15. Overview of the workflow for the BPTMC case study. The process integrates existing information from literature databases (PubMed, Web of Science), the US-EPA CompTox dashboard, and the BPA AOP network, complemented by resources from CIST-ED and SSbD Toolbox of PARC. New data will be generated through in vitro testing of BPTMC and by expanding the bisphenol AOP network. The evidence gathered will support a transparent read-across assessment, guided by EFSA's recommendations and aligned with the bisphenol alternatives project (WP5 of PARC), culminating in the development of the BPTMC case study. ✓ – Performed steps.

Conclusion: Overall, the current body of evidence on BPTMC is still too limited to support a comprehensive hazard assessment. Preliminary findings indicate potential endocrine-disrupting activity and biological effects comparable to other bisphenol analogues, warranting further investigation through targeted experimental studies. The BPTMC case study will contribute to advancing the scientific and regulatory understanding of emerging bisphenol substitutes and their potential implications for human health and developmental toxicity.

8.2 CASE STUDY 2: ASSESSMENT OF THE DNT EFFECTS OF METAL MIXTURES

8.2.1 INTRODUCTION

The toxicological concern for human health posed by a variety of metals, especially heavy metals, is well recognized. They can interfere with multiple body tissues and organs through disruption of basic biological processes (Balali-Mood et al., 2021; Jomova et al., 2025). Four metals that are of particular concern are lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg). They are listed by the World Health Organization (WHO) among the ten chemicals of major public health concern (WHO, 2025). Based on that, Pb, Cd, As, and Hg were selected as the focus chemicals for this case study (Figure 16).

Figure 16. Arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) were the central chemicals investigated in this case study. All four are listed by the WHO as chemicals of major concern (WHO, 2025).

DNT describes adverse effects on the developing nervous system upon chemical exposure. Results can span a variety of outcomes, from structural or phenotypic alterations (e.g., neural tube closure defects, cortex abnormalities, white matter defects) to neurodevelopmental disorders such as autism spectrum disorder (Zhou et al., 2024). While often the links to DNT-causing chemicals are challenging, there is strong epidemiological evidence associating heavy metal exposure of children and pregnant women with adverse neurodevelopmental outcomes (Grandjean & Landrigan 2006, 2014). While also known for their adult neurotoxic capacities, metals such as lead or methylmercury (MeHg; an organic form of Hg) have been recognized for decades to be especially potent in causing DNT. Epidemiological studies from the 1970s onward have shown that lead exposure reduces IQ and contributes to attention deficits and learning problems (Landrigan et al., 1975). Today, there is scientific consensus

that no safe level of lead exposure during brain development exists, as even low-level exposure in children or during pregnancy is associated with adverse effects (Vorvolakos et al., 2016). Awareness was also created after major public health incidents such as the Minamata disease outbreak after MeHg poisoning in Japan in the beginning of the 1950s (Harada 1995). Similar to Pb and MeHg, adverse neurodevelopmental outcomes in children are also reported in epidemiological studies for arsenic (Tyler & Allan 2014) or cadmium (Ciesielski et al., 2012).

Conventional DNT testing relies on animal studies in rodents, such as OECD Test Guideline 426 (OECD, 2007). These studies are rarely performed because they are time-consuming, costly, and only limitedly requested by regulation. Also, they do not provide mechanistic insights. Consequently, a paradigm shift for DNT testing has been proposed in the past decade as a consensus among authorities such as EFSA, OECD, and JRC (Fritsche et al., 2017). A milestone was the development of a DNT in vitro battery (DNT IVB), which has undergone multiple validation steps towards regulatory implementation, culminating in a recently published OECD recommendation document on how to use DNT IVB data (OECD, 2023; Blum et al., 2025a).

To gain further regulatory trust in NAMs (see D2 this report) such as the DNT IVB, case studies with focus on regulatory relevance and questions are required. Due to the complexity of DNT as a toxicological endpoint, mixture risk assessment approaches using NAMs remain rare. In this case study, we address this regulatory need for the exploration of a NAM-based risk assessment strategy for DNT caused by mixtures. Given that Pb, Cd, As, and MeHg are recognized human developmental neurotoxicants within a proposed reference set for evaluating DNT in NAMs (Aschner et al., 2017), they constitute a suitable selection for a case study application.

8.2.2 METHODOLGY AND LINKAGE TO NGRA

Similar to case study 1, we investigated whether a tiered risk-assessment approach is suitable for the case study of DNT metal mixture assessment. In contrast to the data-poor situation for Bisphenol TMC, the general DNT effects of metals are well established and availability of some NAM data from the DNT IVB was expected. Consequently, we aimed for a NGRA-like approach making use of available NAM or in silico data. In recent years, NGRA workflows were

published in different contexts, e.g., as underlying concepts of European toxicological projects (Pallocca et al., 2022; Vinken et al., 2021). NGRA is also key for future approaches of chemical industry to perform risk assessment. Especially, multiple such concepts were developed in cosmetics industry after the ban of animal testing in 2013 (see 6.6. this report; Dent et al., 2021; Baltazar et al., 2020). A potential workflow as used previously by Unilever was presented in the SCAHT webinar series in the talk "*Next Generation Risk Assessment Workflows: The Unilever example"* (see D3, in this report; Figure 17).

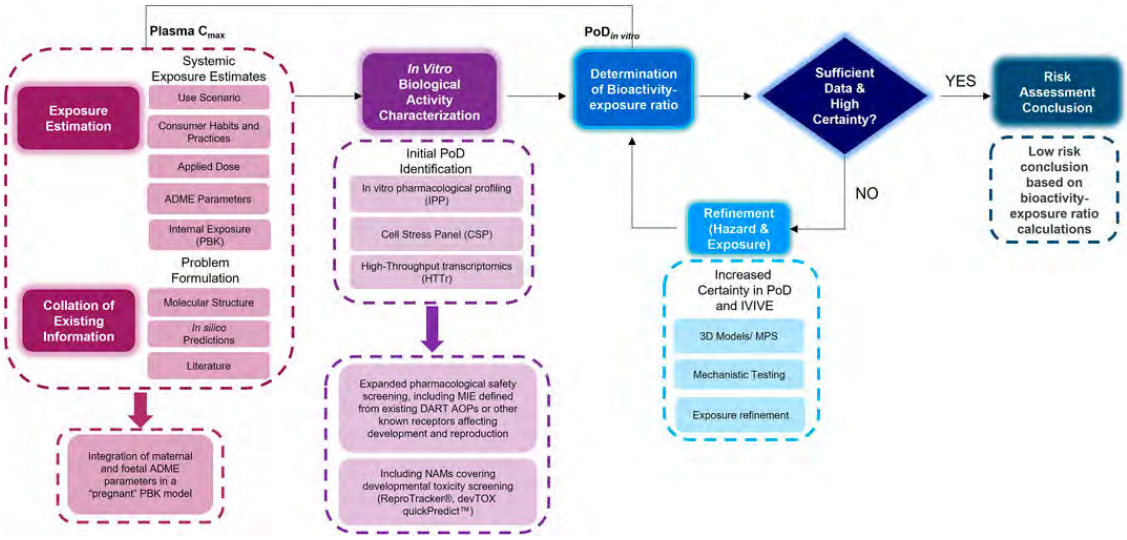


Figure 17. Detailed scheme of a NGRA workflow. An example of a NGRA workflow as presented in the SCAHT Lunch Webinar Series (see D3, in this report). The concept summarizes how Unilever envisions to perform NGRA. Figure from Rajagopal et al., 2022.

The NGRA workflow proposed by Rajagopal et al., (2022) represents a detailed strategy established through many years of development and refinement. The core of the workflow builds on i) the collection of data, ii) the estimation of exposure, and iii) the estimation of hazard in form of in vitro bioactivity data. Ideally, this information results in relationships between exposure levels and hazard PoDs to build the basis to derive risk assessment conclusions by the so-called Bioactivity Exposure Ratios (BERs). The BER is the ratio between the human-equivalent concentration or dose (derived from the in vitro PoD via IVIVE/PBK)

and the estimated human plasma C_{max} or exposure (Rajagopal et al., 2022). In the case of metals, real-life plasma values, e.g. from cord blood studies, might substitute the modelling data as PBK modelling is very difficult for metals.

The aim of the SCAHT case study was not run through such a detailed and complex workflow, but to adapt the general concept to identify a strategy. For this, we orientated on the core aspects of NGRA workflows and developed a simplified NGRA-like scheme for the DNT mixture assessment case study (Figure 18). This NGRA-like workflow starts by the definition of a problem formulation, followed by a (short) scoping of available literature. The next step is the assessment and estimation of exposure levels. For this, we will utilize available human biomonitoring (HBM) data. If possible, we will use Swiss HBM data related to developmental phases, i.e., exposure levels of pregnant women or children. A zero-exposure situation could for example exclude the compounds from a further assessment at this level. In addition, we strive for inclusion of high exposure populations. A step that we integrated in our workflow is the AOP concept. In our workflow, we considered the AOPs as an intermediate step to inform on biological mechanisms and eventually guide hazard assessment.

Figure 18. The workflow developed for the SCAHT case study on metal mixtures. The workflow follows a "NGRA-like" study concept.

For hazard identification using NAMs, we decided to focus on data from the DNT IVB. All steps, starting from literature search to the hazard identification, were planned to be set into the context of potential mixture effects. The ultimate goal was to use the learnings of this NGRA-like workflow to formulate a strategic plan of a detailed NGRA case study on metal mixture DNT effects.

8.2.3 METAL MIXTURE DNT CASE STUDY EXPLORATION AND PRELIMINARY RESULTS

A. Problem formulation

This study aims to assess the feasibility of applying an NGRA-like approach to four metals with known DNT potential and to examine whether the inclusion of mixture toxicology is essential for establishing a robust NGRA workflow.

B. Literature scoping of metal mixture effects

A comprehensive or systematic literature search on the MoAs of all four metals was beyond the scope of this study as this was previously done by others (for example MeHg: Bjørklund et al., 2017; Pb: Tobalu & Enogieru 2025; Cd: Arruebarrena et al., 2023; As: Garg & Bandyopadhyay 2025; five heavy metals: Balali-Mood et al., 2021). These metals in general exert their toxicities, including adult and developmental neurotoxicity, through many different MoAs (Caito & Aschner 2015). Therefore, for this case study, we scoped literature referring to metal mixture effects causing DNT.

In line with this, Karri et al., (2016) reviewed how molecular pathway activation and inhibition by Pb, As, Cd, and MeHg as mixtures can converge to produce cognitive dysfunction (Figure 19). Notably, even when distinct initial pathways are affected (e.g., the glutamatergic system or acetylcholinesterase activity), the downstream consequences may overlap, indicating potential for a variety of combined mixture effects.

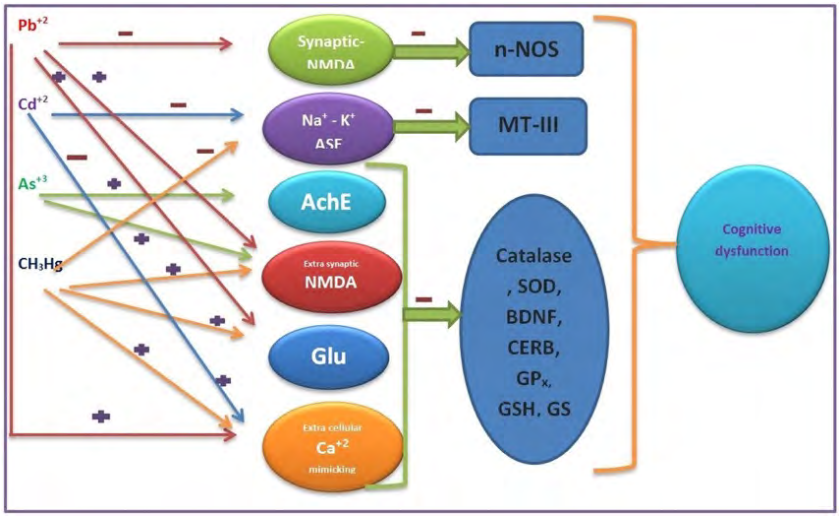


Figure 19. Summary of processes affected by the case study selected metals resulting in cognitive disfunction. Figure from Karri et al., 2016.

Furthermore, studies have reported that certain metal combinations (e.g., As + Cd) can produce greater-than-additive (developmental) neurotoxic effects, consistent with the above-described shared downstream mechanisms (van Stackelberg et al., 2015). A proposed putative general DNT AOP tried to understand mixture effects on a MoA-basis of As, Cd, Pb and Mn earlier. However, MeHg was missing in this work (Figure 20).

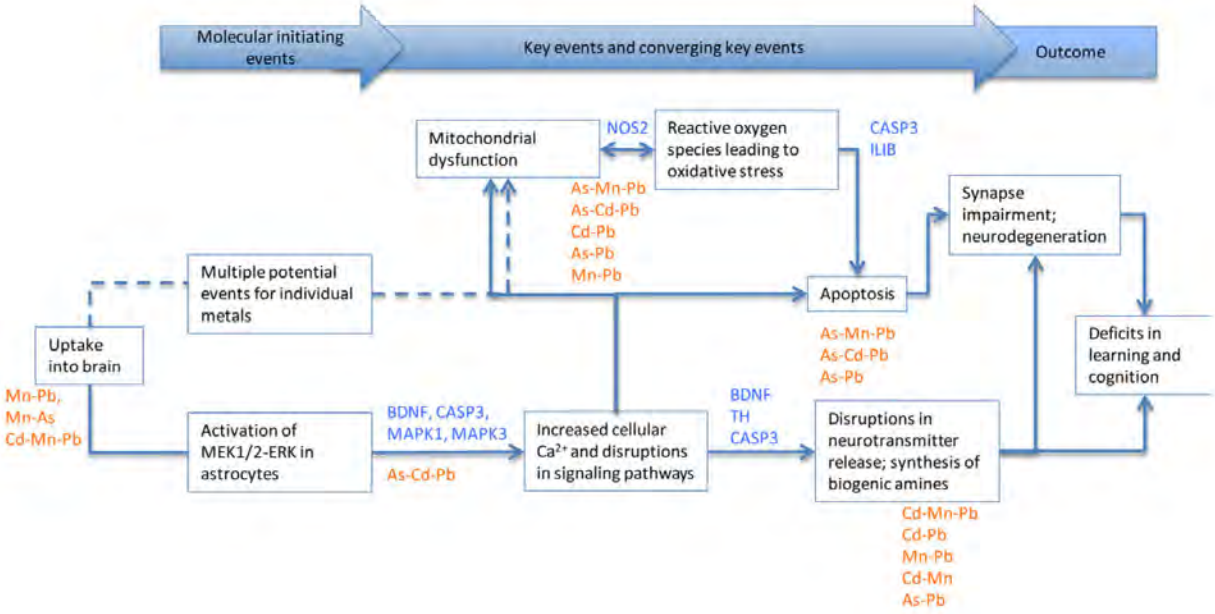


Figure 20. Proposed pathways of potential neurotoxicity of As–Cd–Mn–Pb according to van Stackelberg et al., (2015).

Support for such mixture effects also comes from the key characteristics (KC) for neurotoxicity, which are the outcomes of exposure towards established chemical and biological properties of known human neurotoxicants based on our understanding of their mechanisms of toxicity (Figure 21). These cover adversities on fundamental biological processes such as neurotransmitter disruption, redox imbalance, and dysregulated neuroinflammation. Several of these KC have been reported for at least two of the metals that are included in our study (Lein et al., submitted).

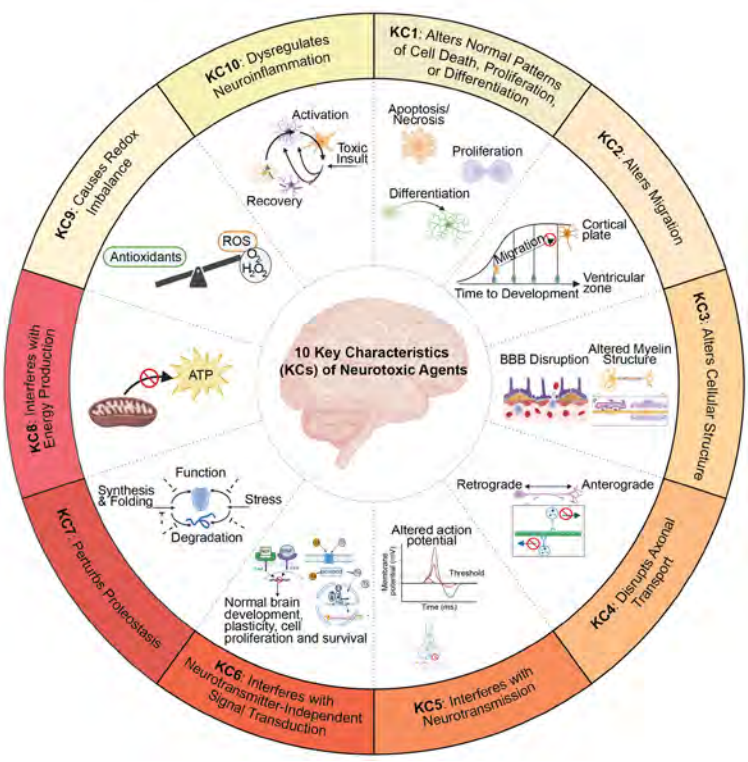


Figure 21. Visual overview of the 10 key characteristics (KC) of neurotoxic chemicals. ATP, adenosine triphosphate; BBB, blood brain barrier; BDNF, brain derived neurotrophic factor; DIO, deiodinase; NGF, nerve growth factor; ROS, reactive oxygen species; RXR, retinoid X receptor; T2, diiodothyronine; T3, triiodothyronine; T4, thyroxine; TR, thyroid receptor; TRE, thyroid response element; Trk, receptor tyrosine kinase (Lein et al., submitted).

Despite the mechanistic/toxicological overlap between the individual metals, an important consideration for mixture toxicity is whether co-exposure to these compounds occurs under realistic conditions. For metals, including As, Cd, Pb, and Hg it is reported that aggregated exposure scenarios are common (Angong et al., 2024). In detail, this means that individuals are exposed via multiple environmental and consumer sources and through different routes (e.g., dietary intake, drinking water, air, soil, dermal contact).

World-wide efforts, with more success among economically strong industrial countries, work on the removal of toxic metals from the environment (Abd Elnabi et al., 2023). In Switzerland, extensive regulatory actions have substantially reduced environmental and consumer exposure to heavy metals over the past decades. The phase-out of leaded petrol and restrictions on mercury and cadmium use, together with remediation of contaminated sites,

have contributed to this decline. The downward trend is also reflected in environmental monitoring data, for instance in decreasing lead concentrations measured in the Rhine River at Basel (BAFU, 2025). Nevertheless, imported goods and certain foods remain potential sources of exposure. An example is seafood (particularly predatory fish), where combined exposure to As and MeHg to the population may occur.

In summary, in addition to individual metals, metal mixture toxicity relevant for DNT remains a concern due to the multiple and overlapping mechanisms contributing to adverse neurodevelopmental outcomes, even though population exposures in Switzerland have markedly declined due to effective regulatory and environmental measures.

C. Exposure assessment – HBM data

HBM data offers the opportunity to get insight into real life exposure levels of society via the measurement of chemicals or biomarkers in human tissues or body fluids. They are valuable data providing direct evidence of internal exposure levels in human populations, helping to bridge the gap between external exposure estimates and actual biological uptake (Reale et., 2024). Such data can serve as indicators for exposures in certain areas of living. For this case study, they may also provide information on exposures of certain subgroups of population, e.g., children for DNT assessment. Furthermore, a potential exposure of multiple metals of interest would support evidence for mixture exposure. We investigated two sources of HMB data, both with measurements of human blood samples.

HBM data from Switzerland: The Federal Council commissioned the FOPH to conduct a pilot study to evaluate the feasibility of a healthy cohort representative of the Swiss population (Swiss Health Study) and to obtain initial data on chemical exposure in the Swiss population. Blood and urine samples were collected from 789 healthy adults aged 20-69 living in the cantons of Bern and Vaud. HBM results from this pilot phase are publicly available in the pilot phase of Swiss Health Study SHeS-pp report (FOPH, 2023). These results are of interest for our case study, as among the investigated compounds were the four metals Hg, As, Cd, and Pb. Data in Figure 22 indicates the 95th percentile of these metals. This exposure indicates a level, where 95% of the participants have exposure levels lower than this value.

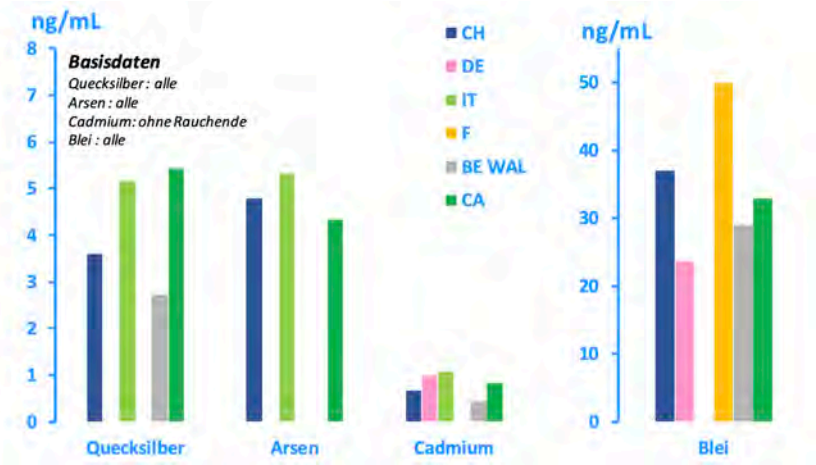


Figure 22. Data from a Swiss HBM investigation on the four metals of interest for the SCAHT case study. Data and figure from pilot phase of Swiss Health Study SHeS-pp report (FOPH, 2023).

From this report, some conclusions can be drawn in respect to our case study:

- In general, the assessed exposure levels of individual metals raised no "exposure concern" in Switzerland. Values of the heavy metals were in general below health relevant limits. Example: The 95th percentile of Hg (total mercury) was 3.6 ng/ml (Figure 22), which is well below the HBM-II (a defined threshold for potential health risk) threshold of 15 ng/ml.

- The values of all four metals in Switzerland (cantons Bern and Vaud) were comparable to neighbouring European countries (Figure 22).

- Cadmium served as an example of aggregated exposure situations, as measured levels were higher among smokers in comparison to non-smoking participants.

- Participants revealed exposure not only to single metals but mostly to all four of them.

In summary, the HBM data provided by the pilot phase of the Swiss Health Study SHeS-pp provides insight for adults from Switzerland. An additional report is available where the exposure to lead of women at the age of procreation was measured (FOPH, 2025). This report may also be used during a follow-up of the case study due to its high relevance for DNT (even though a single metal was assessed).

Given that Swiss exposure measurements were comparable to those reported in European countries and that this report currently includes only adults, we next examined the availability of HBM data for children.

HBM data of neighbouring countries: Based on the assumption, that Swiss population exposure to metals is likely very similar to European countries we assessed further HBM data generated within the HBM4EU project (HBM4EU, 2025). This data does also include samples taken during adolescence and from newborns (Figure 23).

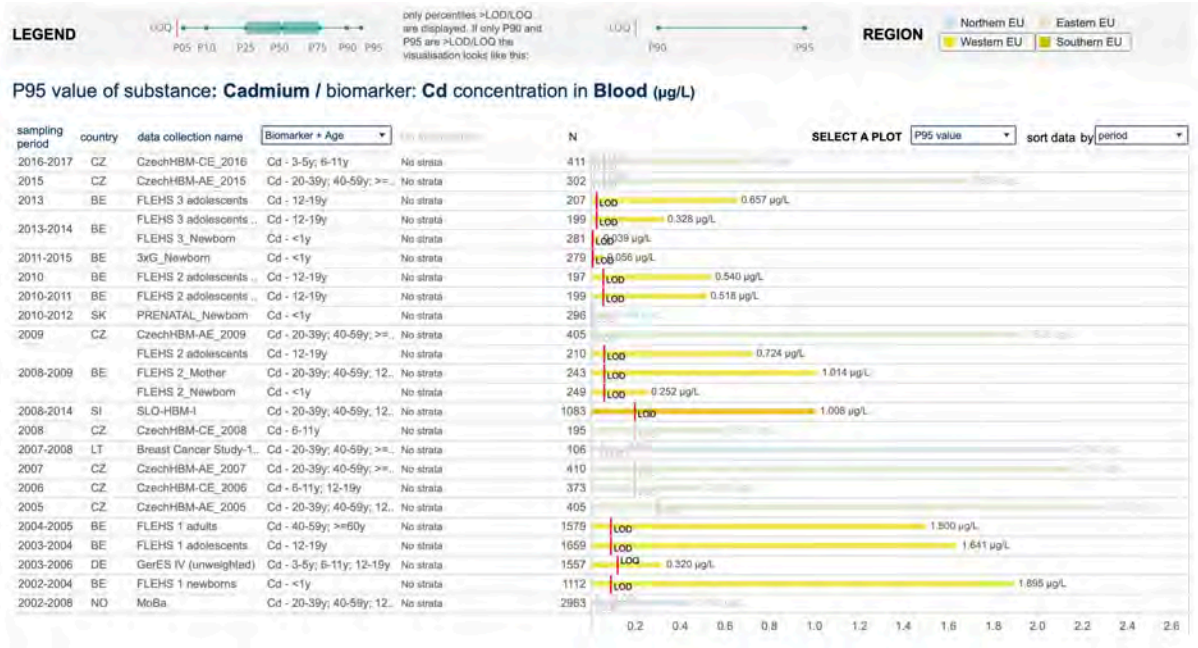


Figure 23. Human biomonitoring data example of cadmium. 95th percentile measured in blood. Data from HBM dashboard (HBM4EU, 2025).

The overview data for cadmium (including newborns <1 year, adolescents, adults; Fig 23 column 4) indicates that besides some fluctuations among sample participants, the 95th percentile values in blood levels for cadmium fall within a range of 0.2 to 2.0 µg/L. This is comparable to what was measured in the pilot phase of Swiss Health Study SHeS-pp (0.7 ng/ml = 0.7 µg/L in Switzerland, for cadmium non-smokers). Notably, the highest 95th percentile identified for cadmium in Western and Southern EU countries in the HBM-Dashboard was obtained in newborns (Figure 23, second row from bottom).

New data in the context of HBM4EU will be available for heavy metals within the PARC project. SCAHT plans to build knowledge on how to access this data and make usage of it. This will also include modelling the data to make it useful in a risk assessment scenario. An option is reverse dosimetry modelling, that can transfer biomonitoring data to exposure scenarios. For this study, we conclude, that HBM data could be used for our proposed workflow. In respect to mixture toxicology, the HBM data confirms that co-exposure to all four metals is likely to occur.

D. AOP linkage - guide mechanistic understanding

AOPs serve as a structured framework to link biological mechanistic events to adverse health outcomes. For NGRA workflows, AOPs can be used as central tools to support scientific plausibility of NAM data and guide decision making. While some well-established NGRA workflows run a default panel of bioactivity assays (see Figure 17, Rajagopal et al., 2022), we considered AOPs also a useful tool to guide mechanistically-relevant NAM data. Vice versa, NAM hazard data from the DNT IVB may be linked to AOPs to increase regulatory relevance (Sachana et al., 2021).

We searched the AOP Wiki database and extracted AOPs that are related to heavy metal stressors and have a DNT-related adverse outcome (AOP-Wiki, 2025). We found three AOPs that have an OECD endorsement status, i.e., that are reviewed by a multi-stage scientific and regularity review coordinated by OECD working parties (Figure 24). Such AOPs are considered trustworthy and reliable. AOP 13 and AOP 12 share the MIE *"chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs)"* and both were constructed based on the toxicity caused by Pb resulting in the adverse outcome (AO) *"impairment in learning and memory"*. An OECD endorsed AOP leading to the same AO is AOP 17. This AOP is initiated by the MIE *"binding to SH-/seleno-proteins that are involved in oxidative stress protection"*. This MIE and the related AOP 17 were constructed referring strongly to the toxicity of MeHg. Other AOPs identified, that are relatively data rich and linked to metals as stressors (but not OECD endorsed) are AOP 500 and AOP 499. For our case study, we used these five high readiness AOPs and investigated potential overlaps in key events to build an AOP-network with the commonality to result in the AO 341 *"impairment in learning and memory"* (Figure 24).

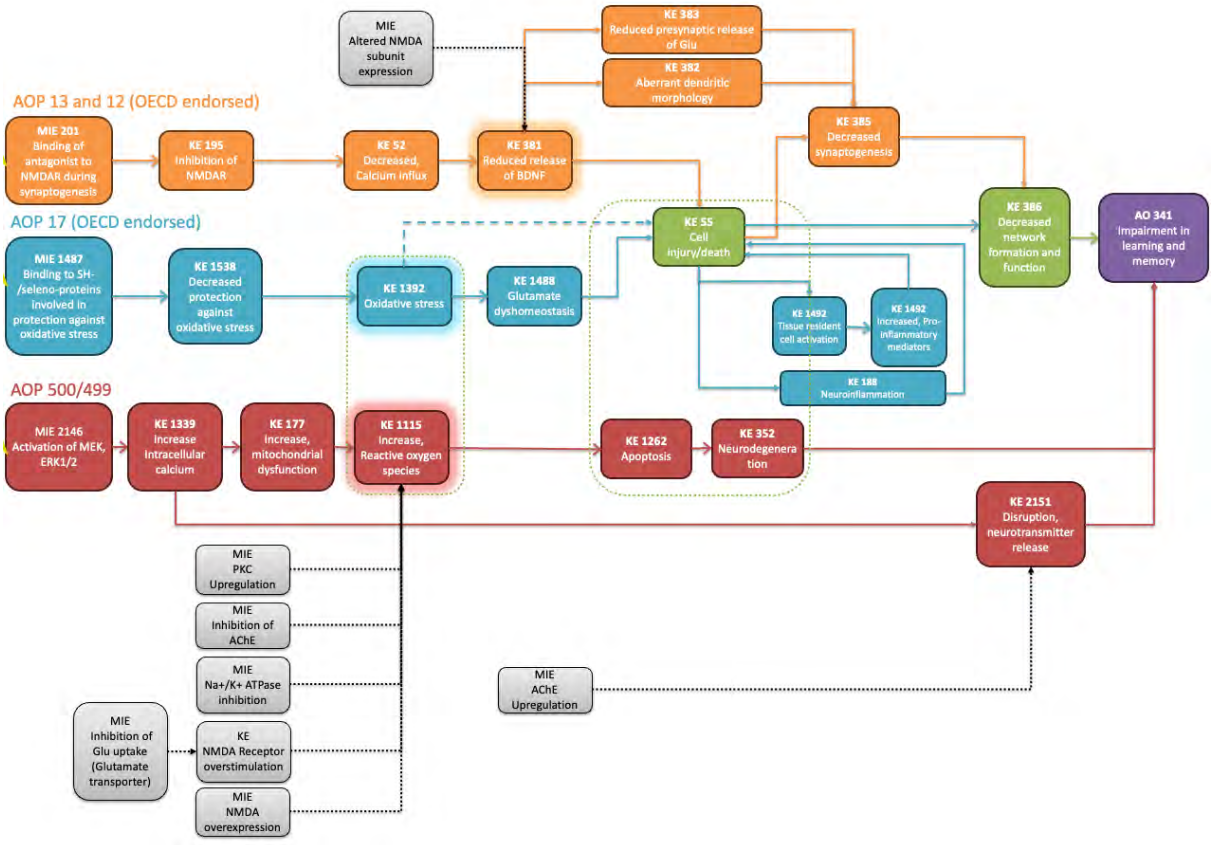


Figure 24. Construction of an AOP network based on AOPs related to heavy metal stressors and the DNT related adverse outcome "impairment of learning and memory". Information was extracted from the AOP Wiki database (AOP-Wiki). Yellow, Blue, Red: Data-rich AOPs. Green: Common key events. Grey: Potential extension of the AOP network based on expert judgement.

Based on expert judgement and our previous literature scoping (see 8.2.3 B), we added further heavy metal stressor-related MIEs and KEs to the network to get an impression on how this network could eventually be expanded (Figure 24, grey boxes).

The generated network encompasses multiple events that align with findings from our literature scoping, notably those associated with the KC of neurotoxicity. An example is the KC 9 "redox imbalance", which is represented by the two related KEs KE1392 and KE1115 (Figure 24). Alterations in calcium levels, NMDA receptor binding, or disturbed neurotransmitter release are further examples of mechanistic events that we found to be reported in the literature (see Figure 19 and Figure 20).

Based on these findings, we assume that in contrast to the single stressor build AOPs, there is a high likelihood that events of this DNT AOP network may be triggered upon heavy metal mixture activity (Figure 25). Besides the MIE, shared key events such as oxidative stress or cell injury may be target sites for mixture toxicity. Here, multiple cell types might be susceptible targets as exemplified by adverse effects of NaAs and MeHg single exposures on DNT KEs earlier (Masjosthusmann et al., 2019; Masjosthusmann et al., 2020; Blum et al., 2023).

Figure 25. Simplified schematic breakdown of the developed AOP network that was built around AOP 17. The AOP knowledge may guide further NAM data extraction from the DNT-IVB. For this we indicated examples where mixture toxicity may occur, the single AOPs share common key events, and where NAMs from the IVB could be used for testing.

We used the AOP-network to guide data extraction of suitable NAMs to cover the events mapped. For a quick overview, we indicated which events are covered in the core assays or in an extended version of the DNTIVB (Figure 25 red stars; Blum et al., 2023; Tal et al., 2024).

E. NAM-based DNT hazard data

To assess NAM-based DNT hazard data we searched for data generated in the assays of the DNT IVB that are currently part of the OECD recommendation (OECD, 2023). These assays have a high level of regulatory readiness and a shared pool of tested compounds for which a large amount of data is publicly available in the ToxCast database (US EPA, 2024d). In addition, our AOP mapping of metal-relevant DNT AOPs revealed many KEs and processes covered within the DNT IVB assays. It is important to note, that even if certain parameters are not directly measured in an assay (e.g., mitochondrial dysfunction assessed via a mitochondrial inhibition assay), many of the KEs highlighted in our AOP network (Figure 24 and 25) may still fall within the applicability domain of individual DNT IVB assays. This means that a downstream consequence of mitochondrial inhibition, such as a reduced cellular energy level, could, for example, result in decreased neuronal migration. These applicability domains are described in detail in the method descriptions of the individual assays (OECD, 2023).

For an initial overview, we investigated whether the DNT IVB detects the metals of interest in our study as hit calls. A general hit pattern profile from a screening of the DNT IVB indicates that all tested heavy metals, including the ones relevant for this case study, showed some level of bioactivity in the DNT IVB assays (Masjosthusmann et al., 2020) (Figure 26).

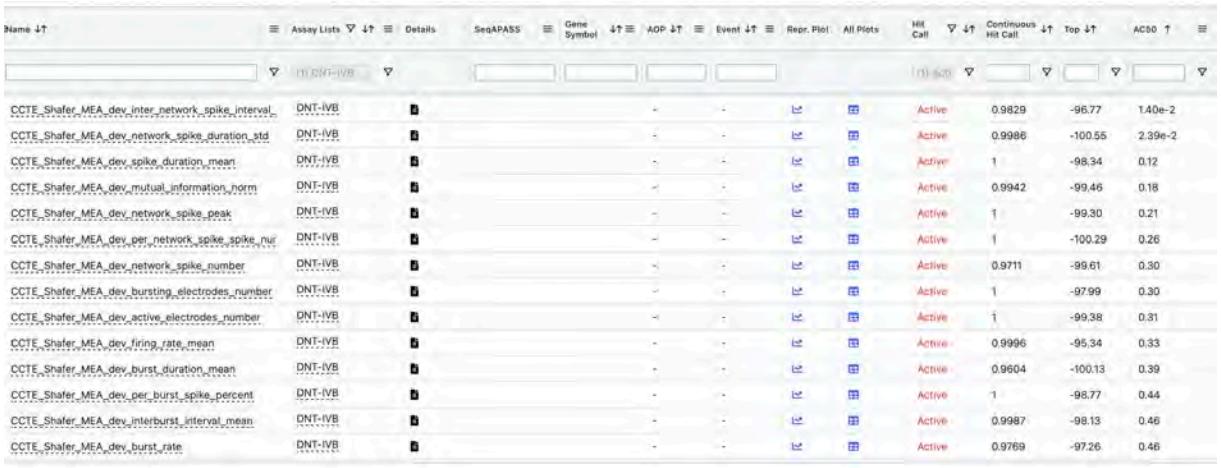
Figure 26. Overview on the activity profile of metals assessed in a screening study of the DNT IVB. Metals of interest for the case study are highlighted. Figure adapted from Masjosthusmann et al., 2020.

Another publicly available source for accessing DNT IVB data is the ToxCast database, which is accessible via the CompTox chemical dashboard (US EPA, 2024d). A first examination of data integrated in the dashboard underscored the importance of further investigating DNT-endpoint-specific NAMs. A comparison for cadmium chloride indicates that the DNT IVB assay

data implemented in ToxCast rank among the most potent biological responses (Figure 27). The mechanistic endpoints showed activities (expressed as the active concentration for 50% response, AC50) occurring well below the general cytotoxic burst, which represents the threshold for nonspecific cytotoxicity (Escher et al., 2020).

Figure 27. A high-level overview on the cadmium data for DNT assays in ToxCast. Single dots represent active concentrations (AC50) for single assays.

The ToxCast Chemical Dashboard enables filtering and ranking of compounds based on defined assay parameters. Using cadmium chloride as an example, we examined which DNT IVB NAM assays were identified as the most potent endpoints, i.e., those with the lowest AC50 values according to the ToxCast data evaluation pipeline. The ranking showed that several Microelectrode Array (MEA) parameters of the network formation assay (NFA), which study a variety of neuronal and neuronal network activity as functional parameters, were among the 15 most sensitive endpoints in the ToxCast database (Figure 28). This finding is particularly noteworthy, as the MEA assay captures neuronal network activity which was identified as a central KE within the constructed DNT-related AOP network (Figure 24 and 25). However, many assays of the DNT IVB have different exposure durations from 24 hours to 12 days of the NFA.



Name	Assay Lists	Details	SeqAPASS	Gene Symbol	AOP	Event	Repr. Plot	All Plots	Hit Call	Continuous Hit Call	Top	AC50
CCTE_Shafer_MEA_dev_inter_network_spike_interval	DNT-IVB				-	-			Active	0.9829	-96.77	1.40e-2
CCTE_Shafer_MEA_dev_network_spike_duration_std	DNT-IVB				-	-			Active	0.9986	-100.55	2.39e-2
CCTE_Shafer_MEA_dev_spike_duration_mean	DNT-IVB				-	-			Active	1	-98.34	0.12
CCTE_Shafer_MEA_dev_mutual_information_norm	DNT-IVB				-	-			Active	0.9942	-99.46	0.18
CCTE_Shafer_MEA_dev_network_spike_peak	DNT-IVB				-	-			Active	1	-99.30	0.21
CCTE_Shafer_MEA_dev_per_network_spike_spike_nur	DNT-IVB				-	-			Active	1	-100.29	0.26
CCTE_Shafer_MEA_dev_network_spike_number	DNT-IVB				-	-			Active	0.9711	-99.61	0.30
CCTE_Shafer_MEA_dev_bursting_electrodes_number	DNT-IVB				-	-			Active	1	-97.99	0.30
CCTE_Shafer_MEA_dev_active_electrodes_number	DNT-IVB				-	-			Active	1	-99.38	0.31
CCTE_Shafer_MEA_dev_firing_rate_mean	DNT-IVB				-	-			Active	0.9996	-95.34	0.33
CCTE_Shafer_MEA_dev_burst_duration_mean	DNT-IVB				-	-			Active	0.9604	-100.13	0.39
CCTE_Shafer_MEA_dev_per_burst_spike_percent	DNT-IVB				-	-			Active	1	-98.77	0.44
CCTE_Shafer_MEA_dev_interburst_interval_mean	DNT-IVB				-	-			Active	0.9987	-98.13	0.46
CCTE_Shafer_MEA_dev_burst_rate	DNT-IVB				-	-			Active	0.9769	-97.26	0.46

Figure 28. Potency of DNT assay data for cadmium chloride in ToxCast. Parameters measured in the MEA assay are the most potent entries.

It is important to note that ToxCast data only based on such dashboard information are not ideally suited for deriving assay-specific PoDs, as the database does not account that individual assay data requires different forms of analysis based on their assay description. Also, many assays of the DNT IVB have different exposure durations from 24 hours to 12 days of the NFA. Moreover, comparison between the DNT-specific endpoint and substance-induced cytotoxicity is not obvious in ToxCast and needs separate analysis. This limits direct comparability across endpoints. A detailed evaluation of the full concentration-response curves is required to define specific DNT hit calls, i.e., cases where distinct endpoints are selectively affected. Such hit calls may also enable mechanistic interpretation based on the nature of the affected endpoint, thereby potentially expanding the existing AOP network.

Consequently, in addition to DNT IVB data retrieved from the ToxCast data base, we examined available peer reviewed DNT IVB assay data for metals. Such data is often not only pure concentration-response data, but integrates mechanistic investigations. Such examples from the published literature of single DNT IVB assays include specific proliferation inhibition of neural progenitor cells (NPCs) by Cd in the NPC1 assay or migration inhibition of NPCs via MeHg (Koch et al., 2022) and specific migration inhibition of neural crest cells (Nyffeler et al., 2017) (Figure 29).

Because these data are based on complete concentration-response relationships, they can also support the derivation of potential PoDs, which could later be applied in risk assessment frameworks. Importantly, IVIVE procedures would be required for this process.

Notably, migration assays showed a particularly strong hit pattern, suggesting that linking these findings back to the relevant AOPs could further refine and expand the network. In fact, all migration assays list inhibition of the electron transport chain (mitochondrial toxicity) as part of their applicability domains (Blum et al., 2023; Koch et al., 2022). However, many different MoA including effects on the cytoskeleton, focal adhesion, autophagy or other cellular signalling, including growth factor signalling, converge on the endpoint migration.

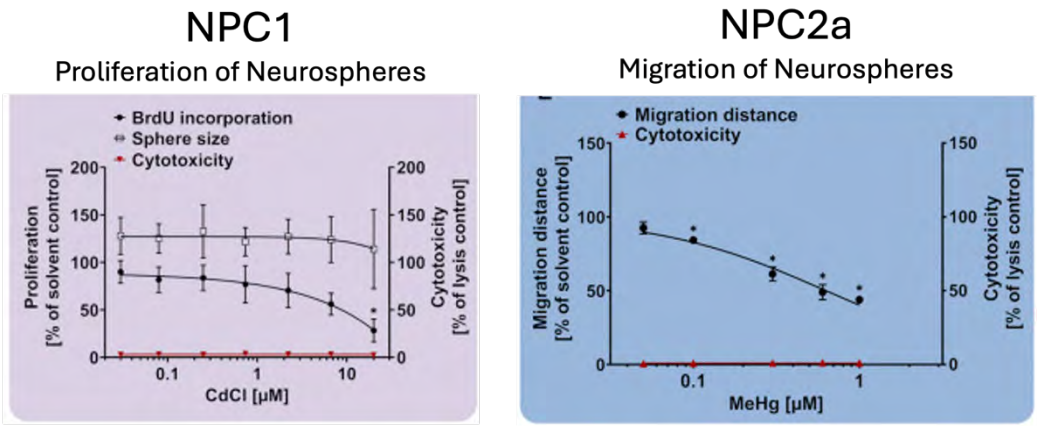


Figure 29. Specific hit calls in the NPC and UKN assays for different heavy metals in publications of assays of the DNT IVB. Data from Koch et al., 2022 and Nyffeler et al., 2017.

Derived learnings from the hazard data exploration are:

- Single metals show effects in multiple assays of the DNT IVB.
- Where metal data is available, the generally most sensitive endpoint seems to be the NFA assay assessed by MEAs. ToxCast data indicates an active concentration of 50% effect level (AC50) for cadmium of 10 nM as the lowest PoD in this assay. However, specificity of these effects will require further detailed investigations.
- The DNT IVB could potentially feed into the AOP-network constructed by confirmation of pathways or by extension/addition of KEs. Especially migration assays of the DNT IVB were identified to have a strong specific inhibitory effect upon metal exposure.
- Specific hit patterns of multiple metals in the same assays could hint to a potential additive mixture toxicity effect. Examples are MeHg and CdCl, which where specific/borderline hits in the UKN2 (neural crest migration), the NPC1 (NPC proliferation) and the NPC2a (NPC migration) assays. However, multiple metal hits on different key neurodevelopmental processes hint to an accelerated adverse effect.
- Published DNT IVB data reveals that metals such as Cd or MeHg are specific hits in more than 4 IVB assays with PoDs in the low micromolar or nanomolar range (Blum et al., 2023).

It was described, that differences in data sources, levels, and handling procedures in all kind of NAMs can alter their outcome and result (Blum et al., 2025b). Especially the impact of different curve fitting data pipelines and resulting alterations in the obtained PoDs has been explicitly showcased using data from the DNT IVB (Carstens et al., 2025; Keßel et al., 2023). Consequently, SCAHT initiated an activity to re-analyse the DNT IVB data in a unified manner using the novel data processing tool CRSTats (DNTOX, 2025).

This tool allows access to ToxCast data and to analyse it in a unified manner. Also, the operator is free to adapt the analysis to the requirements, e.g., adapting where the benchmark response level is set. An example for the NPC2 assay using ToxCast cadmium data is given in Figure 30. This activity is ongoing and was not finalized to date of this report.

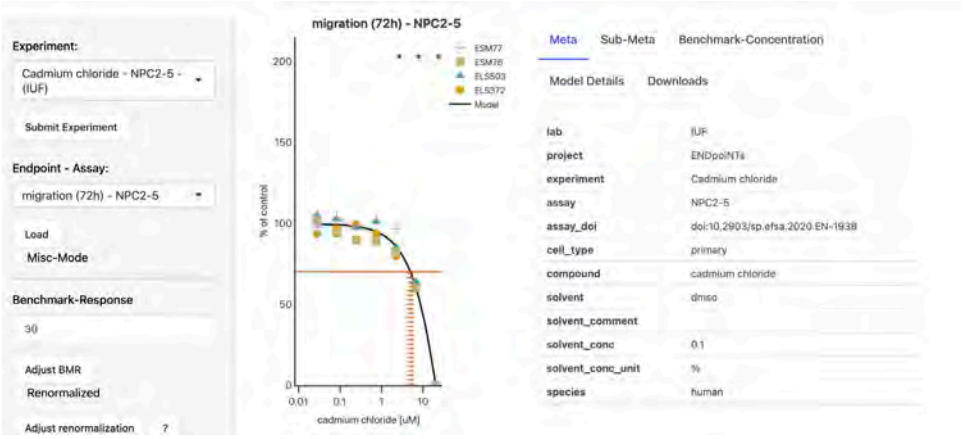


Figure 30. Example of CRStats analysis of cadmium effect on NPC migration in the NPC2a assay. The tool has an active interface that allows to adapt multiple parameters such as the benchmark concentration cut-off.

F. Metal mixture considerations based on the DNT case study

In our NGRA-like workflow, we ensured that each step incorporated considerations of metal mixture toxicity. Overall, we consistently concluded that the likelihood of mixture relevance for DNT induced by metals is high (Figure 31).

The initiation phase of this case study and its multiple assessment levels support the relevance to consider metal mixture toxicity for DNT. Exposure data, including HBM results from Switzerland, indicate that individuals are simultaneously exposed to several metals. Mechanistic insights from available metal-related AOPs reveal shared KEs that can contribute to DNT. Consistently, the NAM-based hazard data from the DNT IVB shows that different metals affect overlapping mechanistic endpoints, reinforcing the potential for combined or additive effects.

Notably, the KCs of neurotoxicity, which will be published later this year (Lein et al. submitted), appear to strongly support the DNT IVB data and the MoA-based AOP framework for evaluating consequences of potential modes of action underlying metal mixture toxicity. Hereby, they serve as a control for verifying neurotoxicological adverse outcomes.

Figure 31. Considerations and learnings on potential metal mixture toxicity that was derived in this case study. All phases explored in the case study support that mixture toxicity should be implemented in a metal related DNT NGRA approach.

To integrate this in a future workflow, in silico tools for mixture toxicity assessment have already been developed and are available. An example is the TKPlate tool developed by EFSA, which was presented during the SCAHT webinar series (see D3, this report; Dorne et al., 2023). However, these tools require more detailed input data than what was collected in this exploratory study, and their application is therefore envisioned as part of a future action plan. Ideally, mixture toxicity assessments should follow international guidance, such as that published by EFSA (EFSA Scientific Committee, 2019).

8.2.4 SUMMARY, OUTLOOK, AND PROPOSED CASE STUDY ACTION PLAN

We developed a conceptual framework outlining the key elements required for conducting an NGRA-like case study. This approach was informed by existing NGRA concepts and was tailored to the capacities and question to address for this case study. In an initial exploratory phase, we applied and tested the proposed strategy by integrating multiple data streams. Specifically, we assessed HBM data to understand population-level exposure to multiple metals, constructed an AOP network based on data-rich AOPs curated in the AOP-Wiki, and investigated approaches for retrieving and integrating DNT IVB assay data to potentially derive PoDs as a foundation for future quantitative risk assessment. Throughout all steps, we systematically examined the relevance of mixture assessment considerations, emphasizing how combined metal exposures may influence both mechanistic interpretation and risk characterization.

As next steps, we propose to apply the strategy explored in this case study, but with more detailed and standardized investigations at each step. To enhance regulatory acceptance but also help identify remaining gaps in the current approach, we propose integrating our proposed workflow (Figure 18) with the recently introduced ASPA workflow (described in 8.1.; Leist et al., 2025; Figure 32). Conversely, as the ASPA framework is still under development, it requires practical case studies to test and refine its structure. We aim to contribute to this effort by serving as one of the first ASPA case studies that explicitly includes mixture risk assessment considerations.

Especially, the ADME module of ASPA should be examined in detail. This would include for example applying DNT optimized IVIVE approaches (Kreutz et al., 2025) to extrapolate in vitro DNT IVB data to human equivalent doses, a step that is a mandatory step in NGRA. A detailed case study will build upon the initial phase of this work and the information for hazard, exposure, and mixtures (highlighted in Figure 32). Future steps will include extraction of DNT IVB data for all metals using the latest laboratory datasets and a unified data-processing pipeline. Data beyond the DNT IVB screening data could be added from other DNT relevant data to not only inform on hazard data on single metals (e.g., As: Masjosthusmann et al.,

(2019)) but also feed into the DNT AOP network (Pistolatto et al., 2020). New mixture data generated by DNT IVB laboratories would allow studying predicted versus real effects.

Figure 32. The recently published concept of the ASPA NGRA workflow. This workflow is intended to make NGRA transparent and applicable to regulators. The ASPA suggests a potential exit for Read-across as it may be applicable for our first case study. For this study, we explored two of the downstream levels and the potential mixture relevance of metals for DNT (red dashed boxes). We suggest to enter the 3 core modules of the ASPA in a detailed study within the European PARC project. Figure adapted from Leist et al., 2025.

Conclusion: This case study by SCAHT explored the feasibility of developing a strategy for a NAMs-based risk assessment of metal mixtures associated with DNT. The proposed approach aligns with the NGRA concept, which was a central theme of the scientific workshop that inspired the present case study (see D3 this report). Our strategy specifically considers Switzerland-relevant aspects, particularly regarding exposure patterns to the selected metals. Where possible, illustrative examples were provided to examine the practical applicability and potential success of the proposed framework. We propose, that a detailed investigation of the presented strategy in alignment with the ASPA workflow within the PARC project could

substantially contribute to a next step in regulatory implementation of NAMs. Specifically, this case study may provide a framework for assessing potential DNT outcomes resulting from metal mixtures under changing future exposure scenarios.

8.3 CONCLUSION ON INITIATION PHASE OF SWISS CASE STUDIES

The D4 report marks an important step forward in advancing Switzerland's contribution to the international implementation of NGRA. Through the two scenario-based case studies on the BPA substitute BPTMC and on the DNT effects of metal mixtures, the project demonstrated how NAMs can be integrated into regulatory contexts with differing data availability and complexity. Both cases illustrate the potential of human-relevant, mechanistic information to complement or, in the long term, replace traditional animal testing while maintaining a robust scientific basis for decision-making.

The initiation phase has shown that the current body of evidence for BPTMC remains limited but provides first indications of endocrine-disrupting activity that warrant targeted in vitro follow-up studies and read-across validation. For the metal-mixture case, a conceptual framework for NAM-based mixture assessment was successfully outlined, linking human biomonitoring, in vitro assays, and AOP networks within the NGRA framework. Together, the case studies exemplify complementary approaches for data-poor and data-rich situations and offer tangible strategies for regulatory implementation.

Going forward, the continuation of these studies under the FOPH - SCAHT collaboration and within European initiatives such as PARC will strengthen Switzerland's position as a bridge between academic innovation and regulatory application. Building on the foundations laid in this report, the next phase will focus on further promotion of the Swiss case studies initiated in D3/D4 by collecting additional mechanistic data, refining the workflows, and enhancing regulatory dialogue to ensure that NAM-based assessments become a credible and accepted standard for chemical safety evaluation.

9 CONCLUSION OF THE OVERALL PROJECT

Over the three-year duration of the project, the SCAHT, in close collaboration with the FOPH, mapped the status quo of NAMs implementation, identified systemic barriers and practical needs, engaged Swiss and international stakeholders through targeted education, and initiated two regulatory-relevant case studies to illustrate future pathways for applying NAMs in real-world decision contexts. Collectively, the findings demonstrate both the significant promise of NAMs and the substantial structural, legal, and cultural challenges that must be addressed before NAM-based NGRA can be integrated into routine regulatory practice.

A comprehensive assessment of the current landscape (D1)

The landscaping exercise (D1) revealed that although risk assessors in Switzerland are broadly familiar with NAMs, their regulatory use remains limited and highly context-dependent. Classical in vivo methods still dominate risk assessment, and NAMs are primarily used in supportive, case-by-case weight-of-evidence evaluations rather than as stand-alone decision tools. Interviews and survey data highlight that this limited uptake does not stem from a lack of scientific potential but from systemic constraints, including prescriptive legal frameworks, reliance on apical animal endpoints, lack of harmonized guidance, resource limitations, and insufficient methodological familiarity among both regulators and industry. Importantly, D1 showed that these challenges are not unique to Switzerland but mirror those encountered across the EU, suggesting that progress requires coordinated international efforts and shared regulatory learning.

System-level barriers, needs, and opportunities (D2)

The gaps & needs analysis (D2) provided a deeper understanding of the systemic, technical, human, and legal barriers preventing broader NAM implementation. Key obstacles include the absence of legally binding frameworks defining how NAM data may replace or complement traditional tests; outdated standard information requirements that exclude mechanistic NAM endpoints; and the lack of harmonized validation approaches aligned with the fit-for-purpose principle. Technical challenges remain substantial, particularly for systemic and chronic toxicity, where current NAMs do not yet fully capture organism-level complexity or toxicokinetics.

Despite these barriers, D2 identified several opportunities for future progress, including expanding the use of read-across, QSAR, PBK modelling, and in vitro test batteries; strengthening early dialogue between developers and regulators and leveraging the growing international momentum for NAM validation and guidance harmonization. These findings informed an action plan outlining SCAHT's role in promoting regulatory uptake of NAMs through targeted training, strategic collaborations, and methodological development.

Building capacity and fostering a shared understanding (D3)

The third project year centred on education, dialogue, and case study selection (D3). A webinar series and an in-person workshop brought together experts from EFSA, Unilever, and other institutions to strengthen Swiss-level knowledge on NGRA, AOPs, IATAs, and PBK modelling. These activities confirmed a strong interest among Swiss agencies in better understanding when NAMs are fit for regulatory use and how mechanistic data can be integrated into structured workflows. Breakout sessions and case study discussions provided valuable insight into the needs and priorities of Swiss authorities, ultimately enabling the identification of two regulatory-relevant case studies to be elaborated in the project's final phase and future initiatives such as the Partnership for the Assessment of Risks from Chemicals (PARC). D3 thereby fulfilled a crucial function in bridging the gap between innovative scientific developments and practical regulatory expectations.

Initiation of Swiss case studies as proof-of-concept (D4)

Two case studies were selected by the FOPH and SCAHT to be developed by SCAHT as D4. The one case study concerns the adverse health effects of Bisphenol TMC (BPTMC) and the second one deals with metal mixtures leading to developmental neurotoxicity (DNT). These D4 case studies illustrate the potential and current limitations of NAM-based workflows in real regulatory scenarios. The BPTMC case study exemplifies a data-poor situation in which literature review, AOP mapping, structural analogues, and read-across approaches offer opportunities for hazard exploration even in the absence of established toxicological datasets. Conversely, the metal mixture DNT case study represents a data-rich yet biologically complex scenario, in which NAMs must be embedded in an NGRA-like workflow to support the assessment of mixture effects.

In both cases, the initiation phase demonstrated promising methodological directions, but also highlighted missing data, incomplete assay coverage of key events, and the need for enhanced model integration and toxicokinetic considerations. As such, both case studies underscore the central conclusion that NAMs can meaningfully inform regulatory risk assessment, but that solid data bases embedded in frameworks like NGRA or IATA are needed before they can drive decisions.

Overall conclusions and implications for Switzerland

Taken together, D1 - D4 show that the scientific foundation for NAMs is rapidly advancing, and international regulatory bodies increasingly recognize their value for mechanistic, human-relevant risk assessment. However, the transition from traditional toxicology to NGRA remains constrained by legal rigidity, uneven global acceptance, limited methodological readiness for systemic toxicity, and insufficient alignment between scientific innovation and regulatory practice. The project highlights the importance of coordinated international collaboration, harmonized guidance, and a paradigm shift toward fit-for-purpose validation rather than one-size-fits-all requirements.

For Switzerland, the dual effort of aligning with EU frameworks while advancing its own scientific capabilities allows SCAHT and the FOPH to assume a pivotal bridging role in supporting the regulatory acceptance of NAMs. Through targeted training, strategic foresight, and continued engagement in PARC and OECD initiatives, Switzerland can strengthen regulatory familiarity with NAMs, build scientific confidence, and promote the use of mechanistic evidence in risk assessment. The initiated case studies provide a scalable foundation for this future work, demonstrating both feasibility and the need for iterative refinement.

Outlook

This project represents a significant step toward embedding NAMs within regulatory practice in Switzerland. It generated a detailed understanding of current barriers and opportunities, built capacity among risk assessors through structured knowledge exchange, and initiated practical applications through two regulatory-relevant case studies. Continued progress will require expanding empirical data, advancing mechanistic modelling, integrating

toxicokinetics more systematically into NAM workflows, and establishing clearer regulatory pathways for the acceptance of NAM-generated evidence. As international momentum accelerates, including forthcoming European roadmaps and global harmonization efforts, Switzerland is well positioned to contribute to and benefit from this transformative shift toward more efficient, ethical, and human-relevant risk assessment.

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