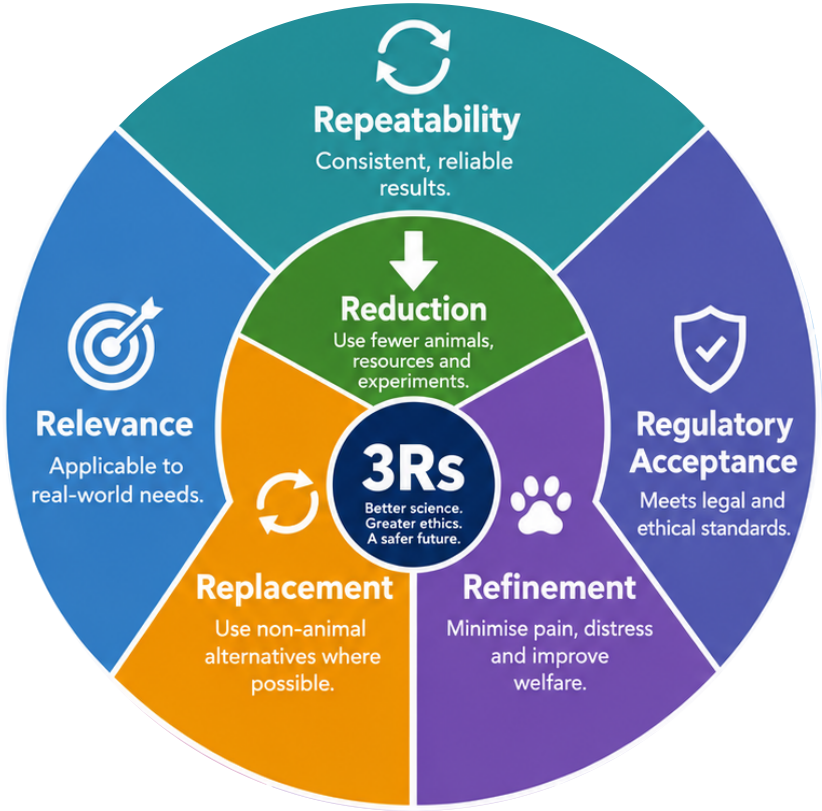
The Rise of New Approach Methodologies (NAMs) for Environmental Safety Assessment of Chemicals: Current Status, Challenges and Strategies for Broader Adoption of NAMs

Adam Lillicrap





The 3Rs

In This Issue:

ET&C FOCUS

Focus articles are part of a regular series intended to sharpen understanding of current and emerging topics of interest to the scientific community.

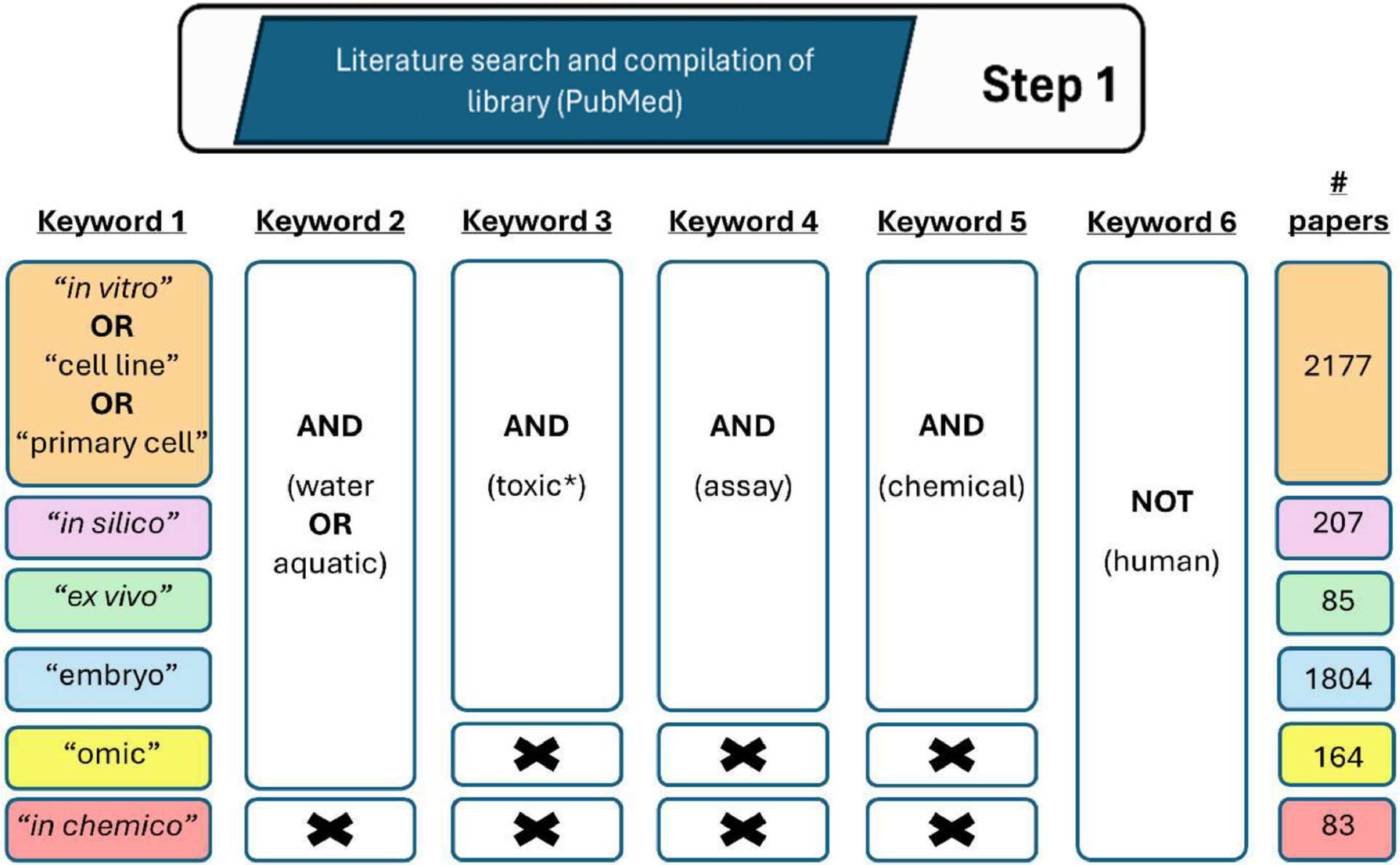
Alternative Approaches to Vertebrate Ecotoxicity Tests in the 21st Century: A Review of Developments Over the Last 2 Decades and Current Status

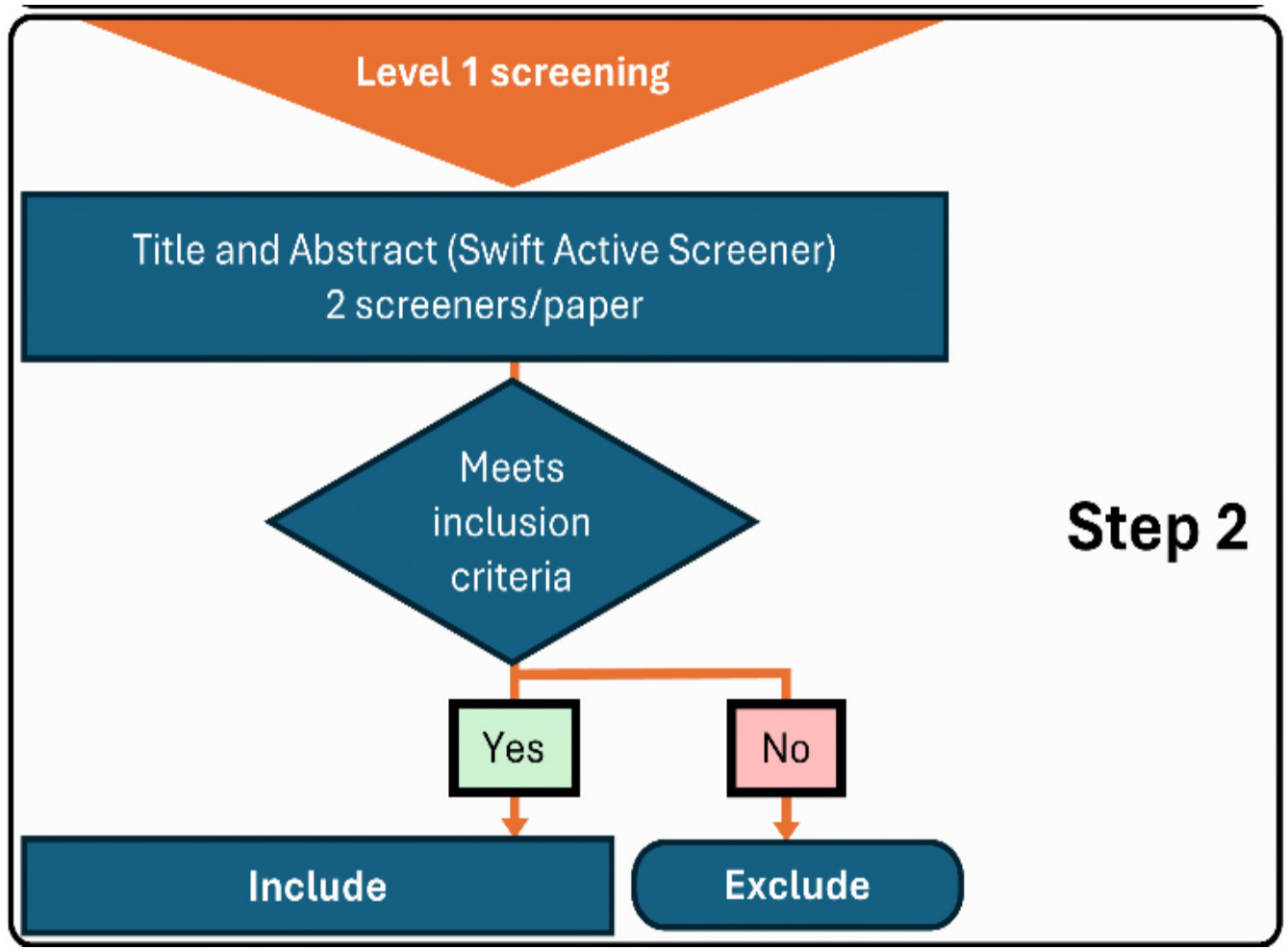
Adam Lillicrap,*† Scott Belanger,‡ Natalie Burden,§ David Du Pasquier,‖ Michelle R. Embry,# Marlies Halder,†† Mark A. Lampi,‡‡ Lucy Lee,§§ Teresa Norberg-King,‖‖ Barnett A. Rattner,## Kristin Schirmer,†††‡‡‡§§§ and Paul Thomas###



The evolution of the 3Rs

Systematic review process

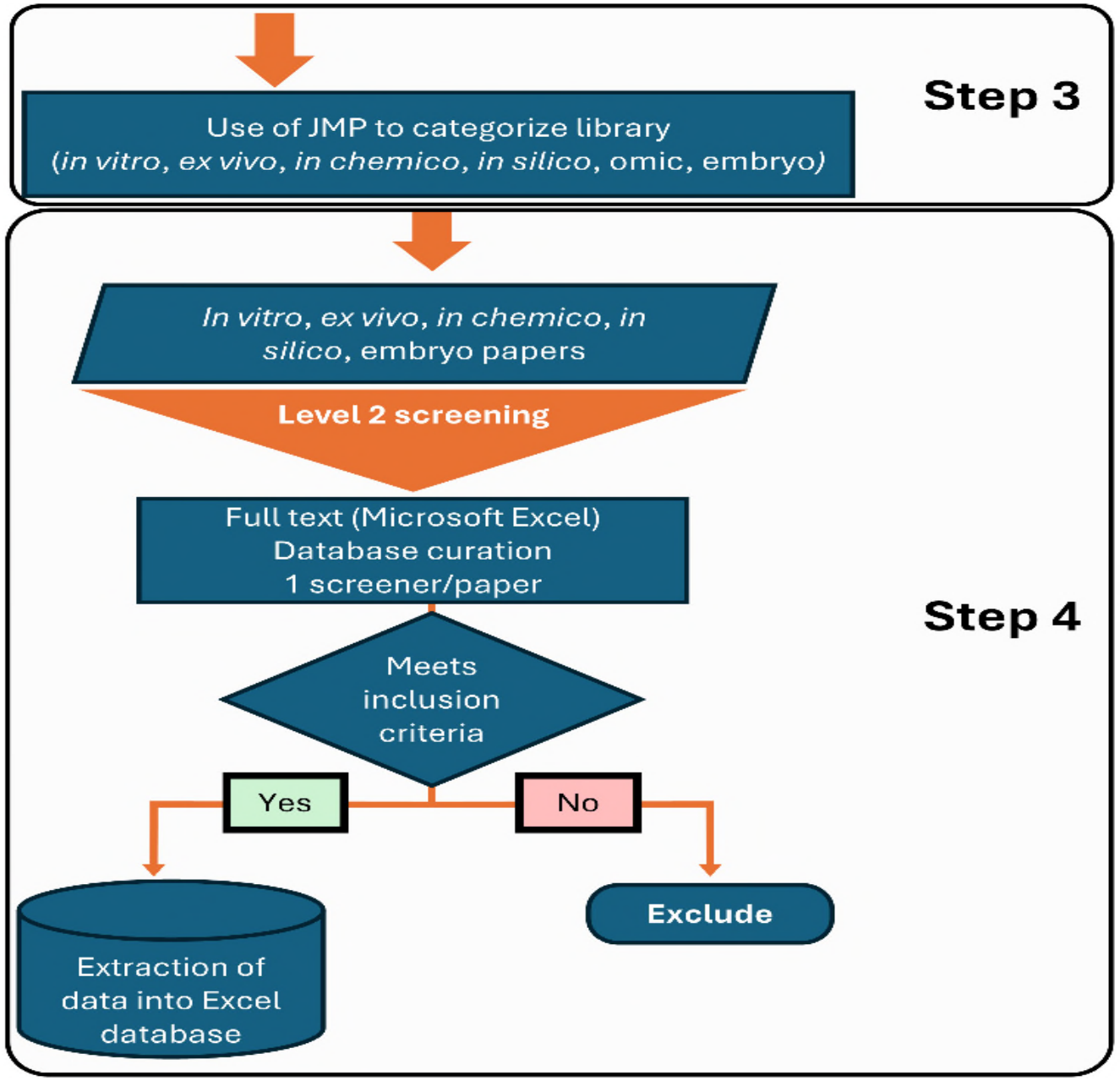



Level 1 screening
Title and Abstract (Swift Active Screener)
2 screeners/paper
Meets inclusion criteria
Yes
No
Include
Exclude
Step 2

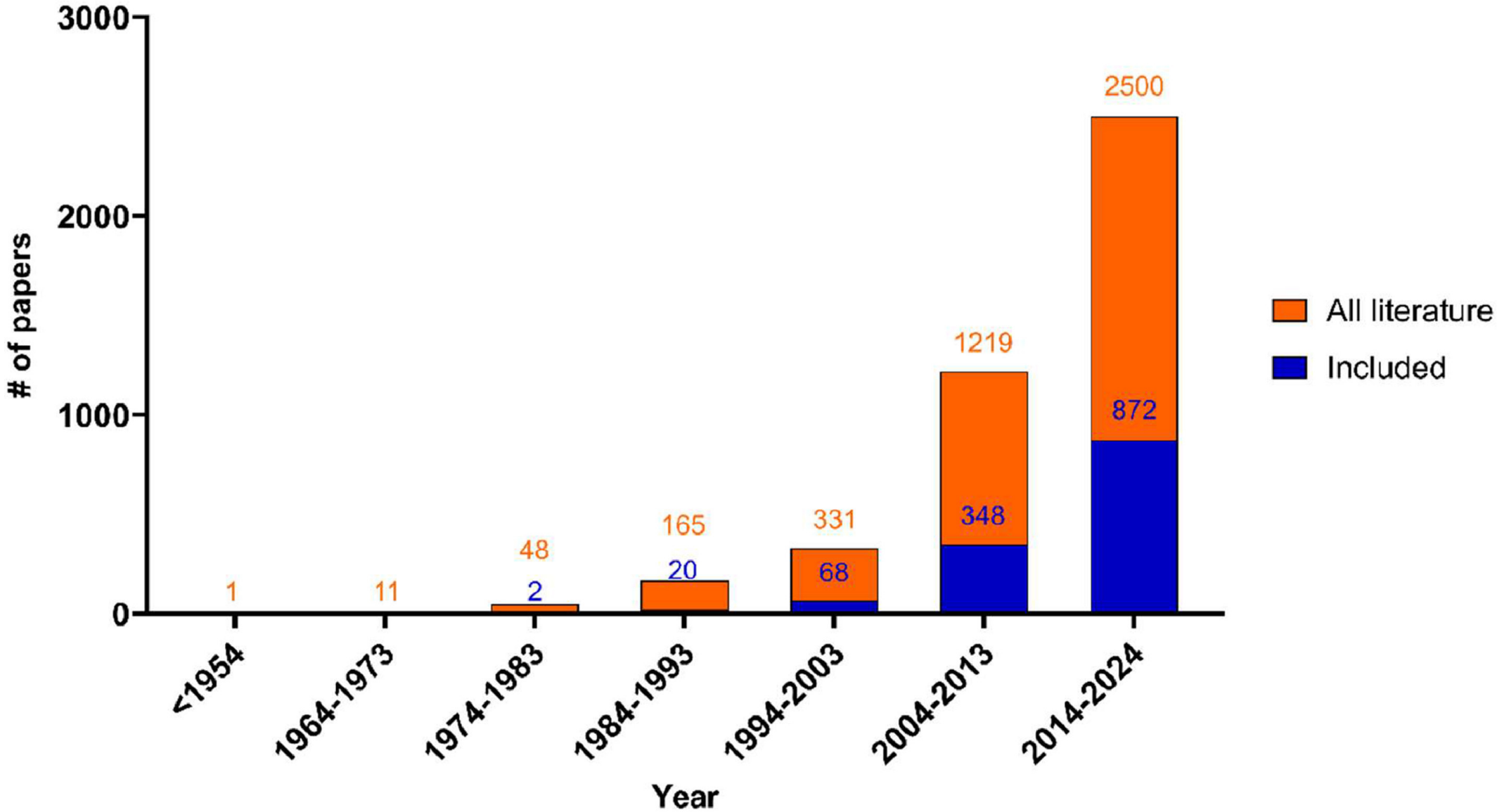
NIVA ECOTOX

Inclusion Exclusion criteria

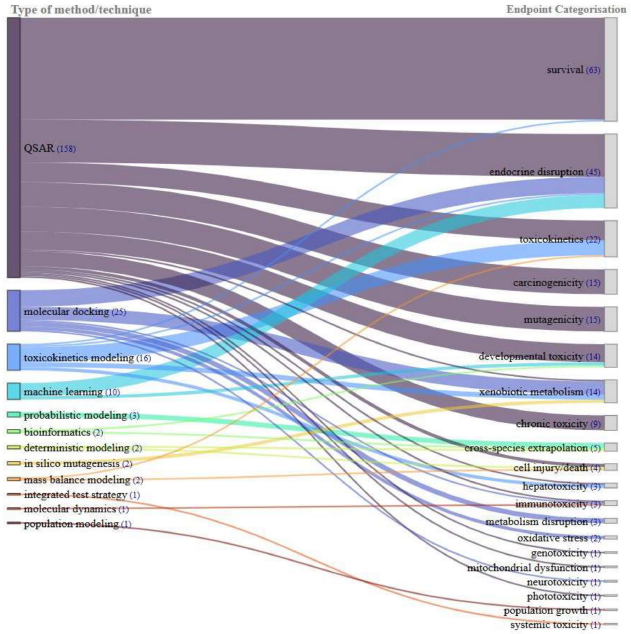
Inclusion	Exclusion
•In vitro exposure and assays (fish, amphibians and marine mammals); •Embryo exposure irrespective of species if the exposure doesn't exceed the endogenous feeding stage; •Embryo exposures if they exceeded the non-protected life stage but only if they evaluated endpoints within the non-protected life stage period; •In silico approaches using in vivo data (both existing and experimental) if relevant species (i.e., fish, amphibians, marine mammals); •Exposure of chemicals using microinjection; •Scientific papers written in English; •Fish receptor transfection or binding assays.	• In vivo exposures followed by in vitro assays; •In vitro studies with non-aquatic mammalian cells (i.e., human, mice, rats); •Generation or offspring effect(s); •Early life stage/whole life cycle exposures; •Embryo papers when protected life stages were exposed to chemicals. •Yeast estrogen and androgen screening assays; •Studies using bacteria, algae, birds or invertebrates; •Reviews, commentaries or concept papers; •Book chapters; Restricted or subscription only scientific papers; •Methodological papers, i.e., characterization of cell lines, optimization of biological assays without further chemical exposure; •Embryo papers that investigated the effects of chemicals only on traditional toxicity endpoints such as survival and morphology (malformations).

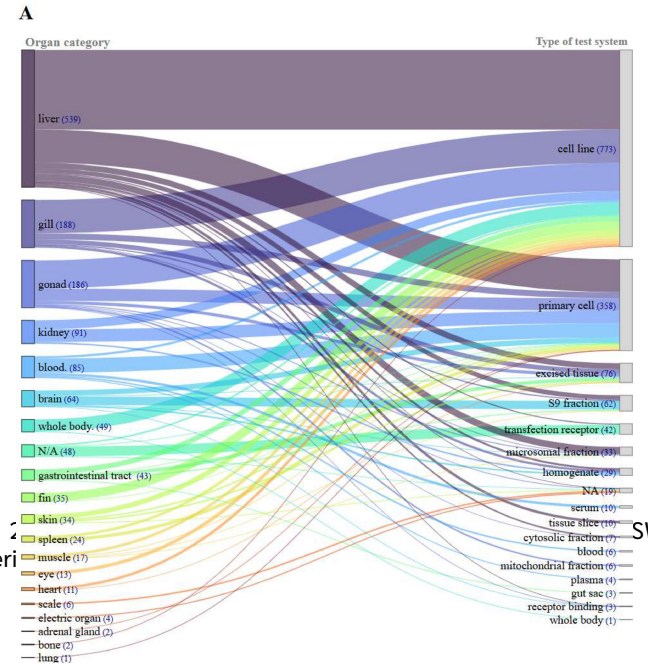


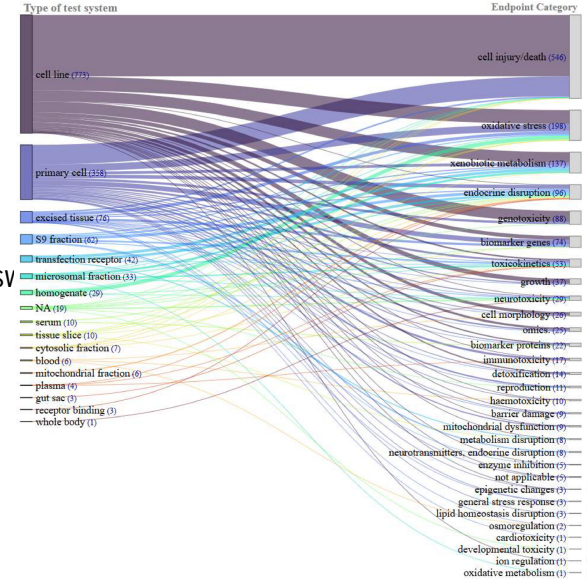
Screening identified 4,275 unique articles; 1,310 included for full-text review

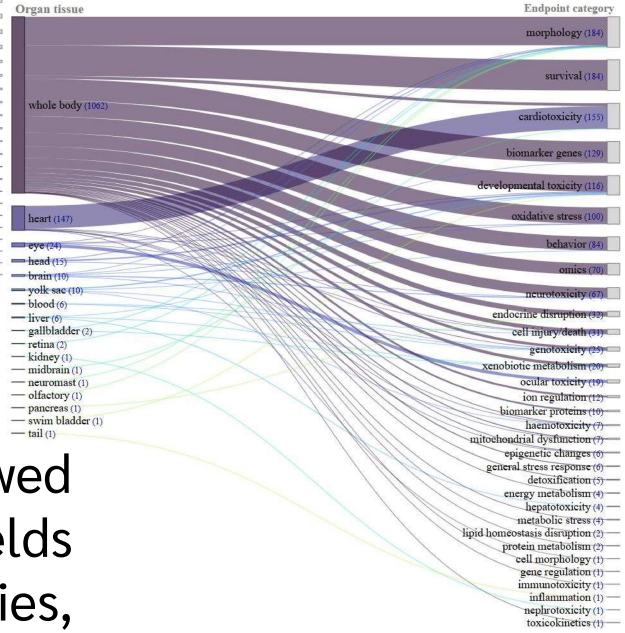

3000
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331
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1219
348
2500
872
All literature
Included
<1954
1964-1973
1974-1983
1984-1993
1994-2003
2004-2013
2014-2024
Year

Sankey diagrams









Published April 24, 2026 | Version 1

Dataset | Open

The Rise of New Approach Methodologies (NAMs) for Environmental Safety Assessment of Chemicals: A Systematic Review of the Current Status, Challenges, and Strategies for Broader Adoption of NAMs

Christou, Maria (Contact person)[1]; Georgantzopoulou, Anastasia (Contact person)[1]; Hultman, Maria T. (Data collector)[1]; Song, You (Data collector)[1]; Wennberg, Aina Charlotte (Data collector)[1]; Mentzel, Sophie (Data collector)[1]; Embry, Michelle (Data collector)[2]; Ott, Amelie (Annotator)[3]; Suzuki, Takahiro (Annotator)[4]; Grassi, Giacomo (Annotator)[5]; et al. Show all 12 authors

Contributors

Data collector: Belanger, Scott

When developing the database, information from each paper was added to 18 columns of the Excel database (Ref to ATEST database to be included here). These 18 columns were: Inclusion, Type of NAM, Taxa, Species, Sex, Life stage, Organ/tissue, Type of test system, Cell type, Cellosaurus ID, Commercial cell line (Y/N), Commercial Accession number, Source, Cell line name, Type of method/technique, Method name, Endpoint (target) and Endpoint categorisation. Not all columns were applicable for the embryo screening, so the columns were reduced to Inclusion, Type of NAM, Taxa, Species, Organ/Tissue, Type of method/technique, Method name, Endpoint (target) and Endpoint categorisation.

Files

Files (2.3 MB)

Name	Size	
Database categorised_250115_20260312.xlsx md5:6d8baea45e92ecb00ca53084d9f5733a	2.3 MB	Download

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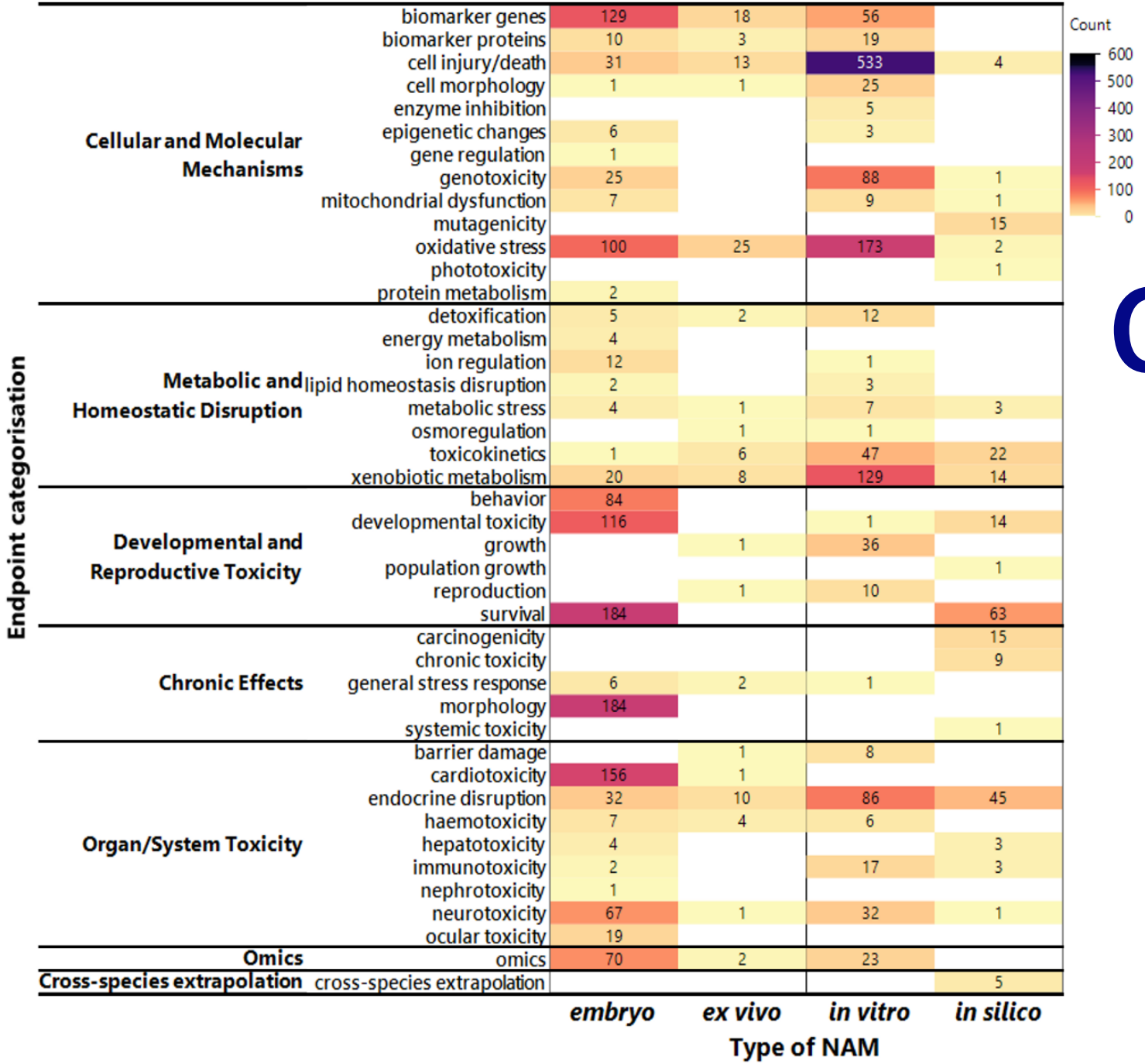
Keywords and subjects

3Rs | NAMs | systematic literature review | animal alternatives

Database developed with all reviewed information containing 18 metadata fields including the assay type, species, endpoints, and readiness level.

Proposed NAMs readiness levels

NAM readiness level (NAMRL)	Description of the level of readiness	Examples of different methods at different levels of readiness from reviewed literature database
NAMRL 1	Basic principles developed and investigated	Co-culturing *in vitro* test systems incorporating different cell types; High-content imaging; Precision-cut tissue slices (PCTS)
NAMRL 2	Experimental method formulated (standard operating procedure)	Multi-OMICS point of departure/moPOD; G2PScan
NAMRL 3	Experimental proof of concept	*Ex vivo* gut sacs; Transactivation assays; Transcriptomic point of departure/tPOD; SeqAPASS
NAMRL 4	Method validated in 1 laboratory (intra laboratory validation)	*In vitro* 3D hepatic spheroid intrinsic clearance rate assay
NAMRL 5	Method transferred successfully to multiple laboratories	Comet assay (*in vitro*)
NAMRL 6	Method validated in an interlaboratory ring trial	Behaviour assays for developmental neurotoxicity (zebrafish embryos using video tracking software)
NAMRL 7	Method demonstrated to standardized organization (e.g. OECD)	Inclusion of thyroid endpoints in OECD fish tests
NAMRL 8	Method completed and approved by standardization organization	OECD 319 (hepatic biotransformation); FET test (OECD 236); RTgill-W1 cytotoxicity assay (OECD 249)
NAMRL 9	Method accepted for regulatory test requirements (e.g. EU REACH)	EU REACH: *Hyallela azteca* bioaccumulation test (HYBIT- OECD 321)



GAP analysis

Recommendations & Conclusions

- Accelerate interlaboratory ring trials and SOP development and expand OECD Test Guidelines to include mechanistic endpoints;
- Develop advanced models (e.g. co-culture systems, 3D organoids, microphysiological systems) and prioritize developing NAMs for poorly covered endpoints (e.g. immunotoxicity and epigenetics);
- Establish workflows for transcriptomic/metabolomic points of departure (tPOD, mPOD) to enable quantitative risk assessment;
- Link NAM endpoints to AOPs for mechanistic interpretation and regulatory relevance;
- Combine NAMs within IATAs for weight-of-evidence decision-making;
- Promote cross-species extrapolation and IVIVE modeling for ecological relevance;
- Align aquatic NAM strategies with human health frameworks to leverage synergies.

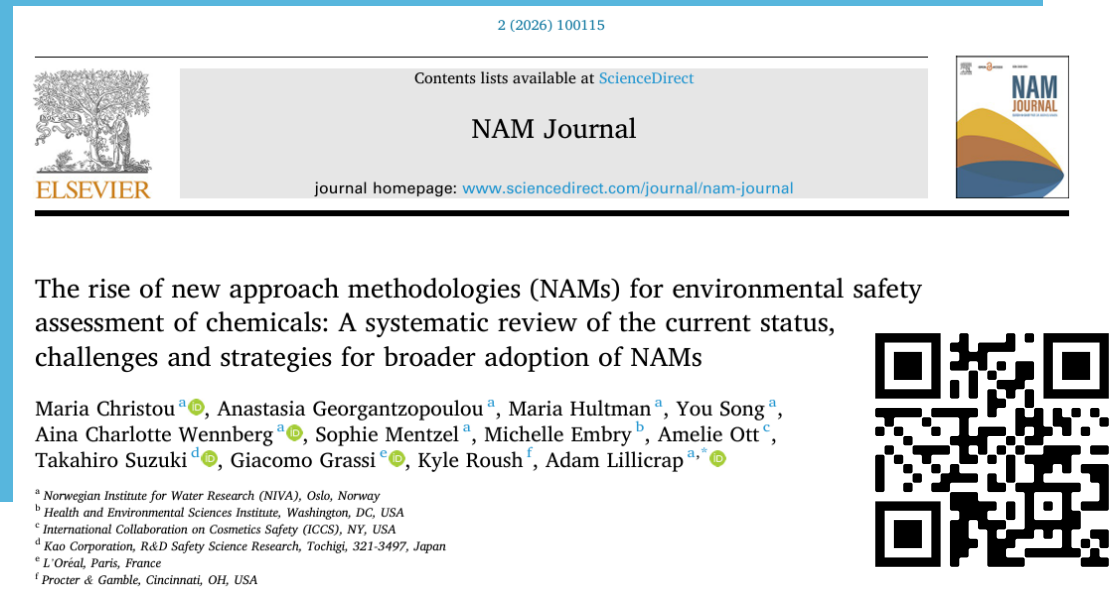
Success depends not on replacing individual animal tests one-for-one, but on building confidence in integrated NAM-based decision frameworks that are fit-for-purpose for environmental risk assessment and aid **Avoiding** vertebrate testing wherever possible.

Acknowledgements

- M Christou, A Georgantzopoulou, M Hultman, Y Song, AC Wennberg, S Mentzel, M Embry, A Ott, T Suzuki , G Grassi and K Roush
- Thanks to S Belanger, B Campos and R Heisler

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Maria Christou [a], Anastasia Georgantzopoulou [a], Maria Hultman [a], You Song [a], Aina Charlotte Wennberg [a], Sophie Mentzel [a], Michelle Embry [b], Amelie Ott [c], Takahiro Suzuki [d], Giacomo Grassi [e], Kyle Roush [f], Adam Lillicrap [a,*]

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