Environmental Science and Ecotechnology 28 (2025) 100638

Contents lists available at ScienceDirect

Environmental Science and Ecotechnology

journal homepage: www.journals.elsevier.com/environmental-science-and-ecotechnology/

Commentary

Beyond animal testing: An integrated framework for modern chemical hazard identification and risk assessment

The report "Making Peace with Nature: A scientific blueprint to tackle the climate, biodiversity and pollution emergencies," released by the United Nations Environment Programme, highlights that the Earth is facing three major crises: climate change, biodiversity loss, and environmental pollution [1]. The *Lancet* Commission on pollution and health stated that 9 million people, representing one-sixth of global fatalities, died from pollution in 2019, among which more than 1.8 million deaths were caused by toxic chemical pollution [2]. Since the 21st century, chemical management and emerging contaminants have received unprecedented attention due to the rapid growth in the number and types of pollutants detected in the environment. Animal toxicity testing is a crucial method for revealing the toxicity mechanisms of chemicals and safeguarding human health and ecological security. However, such a method is critically constrained by a prolonged testing period, exorbitant costs, and low throughput. Therefore, the growth rate of chemicals far exceeds the testing capacity of animal-based methods. By the end of 2024, globally registered chemicals had reached 279 million, while fewer than 20,000 had undergone toxicity assessments [3]. A large number of chemicals are trapped in toxicity data gaps, which will further lead to the delay in their assessment and regulation. Meanwhile, emerging pollutants exhibit more complex toxicity mechanisms than traditional pollutants, such as non-monotonic dose–effect relationships, multi-target synergies, and high risks associated with chronic, low-dose exposures. Additionally, several traditional pollutants exhibit unusual toxic characteristics and require reevaluation. For example, natural organic matter can generate numerous carcinogenic disinfection byproducts during the chlorination process of source water, posing a threat to human health [4]. The surge in the number of chemicals, the limitations of animal testing, and the more complex toxic mechanisms of pollutants pose challenges to environmental toxicology research and public health. It is necessary to explore new approach methodologies (NAMs).

The current NAMs focus on non-animal technological pathways and involve multi-disciplinary technologies, including in chemico, in vitro, and in silico approaches [5]. These NAMs can achieve rapid, accurate, and high-throughput assessment of chemical toxicity. As of November 2024, the United States ToxCast/Tox 21 project had generated numerous biological target and activity data for over one million chemicals using high-throughput in vitro assays, increasing the screening efficiency of chemical toxicity by approximately three orders of magnitude compared with animal testing [6]. Furthermore, NAMs can more accurately identify species-specific differences in chemical toxicity, compensating for the toxicity deviations in animal testing. A related study reported that multispecies liver chips (comprising mice, dogs, and humans) accurately predicted liver toxicity and identified species differences and human risks associated with drugs by providing multiple toxic endpoints [7]. However, the previous research focused on piecemeal applications of NAMs. Advancing the field requires a comprehensive interdisciplinary understanding that spans chemistry, toxicology, computer technology, biology, and environmental science. Thus, a unified and coherent framework that integrates disparate NAMs has yet to be established.

Establishing a non-animal framework based on NAMs is not only a significant strategy to address current scientific challenges but also a crucial support for advancing the assessment and regulation of global chemicals. Ethical controversies of animal testing, together with the 3R principles—reduction, refinement, and replacement—continue to gain prominence. The 3R principle advocates for reducing animal use, refining experimental conditions to minimize animal suffering, and replacing in vivo experiments with alternative methods. Currently, some countries or organizations are promoting new alternative methods. The European Union has comprehensively banned animal testing in cosmetics and applied Organisation for Economic Co-operation and Development (OECD)-certified in vitro models [8]. In 2022, the United States Food and Drug Administration launched an agency-wide New Alternative Methods Program to support the legal application of NAMs in the product supervision system [5]. The Weight-of-Evidence evaluation that uses multiple non-animal methods is accepted for cosmetics or pesticides in South Korea, Japan, and Canada [8]. The National Institutes for Food and Drug Control in China is considering four in vitro tests involving eye and skin toxicology in cosmetic safety assessment. Overall, a limited number of NAMs are incorporated into regulatory settings, primarily applied in the assessment of cosmetics, pharmaceuticals, and food. The assessment and regulation of most chemicals need animal testing. The regulatory approval of NAMs largely relies on their validation and standardization. Therefore, establishing a non-animal framework based on NAMs is essential for enhancing their applicability and accelerating regulatory acceptance.

Under the dual demands of scientific research and regulatory supervision, we propose a non-animal framework comprising three modules: high-throughput data screening, toxicity identification driven by multi-source data, and risk evaluation based on in vitro validation and optimization (Fig. 1).

Firstly, a high-throughput classification, filling, and screening process should be adopted to determine priority toxic chemicals. Congeneric contaminant groups and their toxicological priority

https://doi.org/10.1016/j.ese.2025.100638

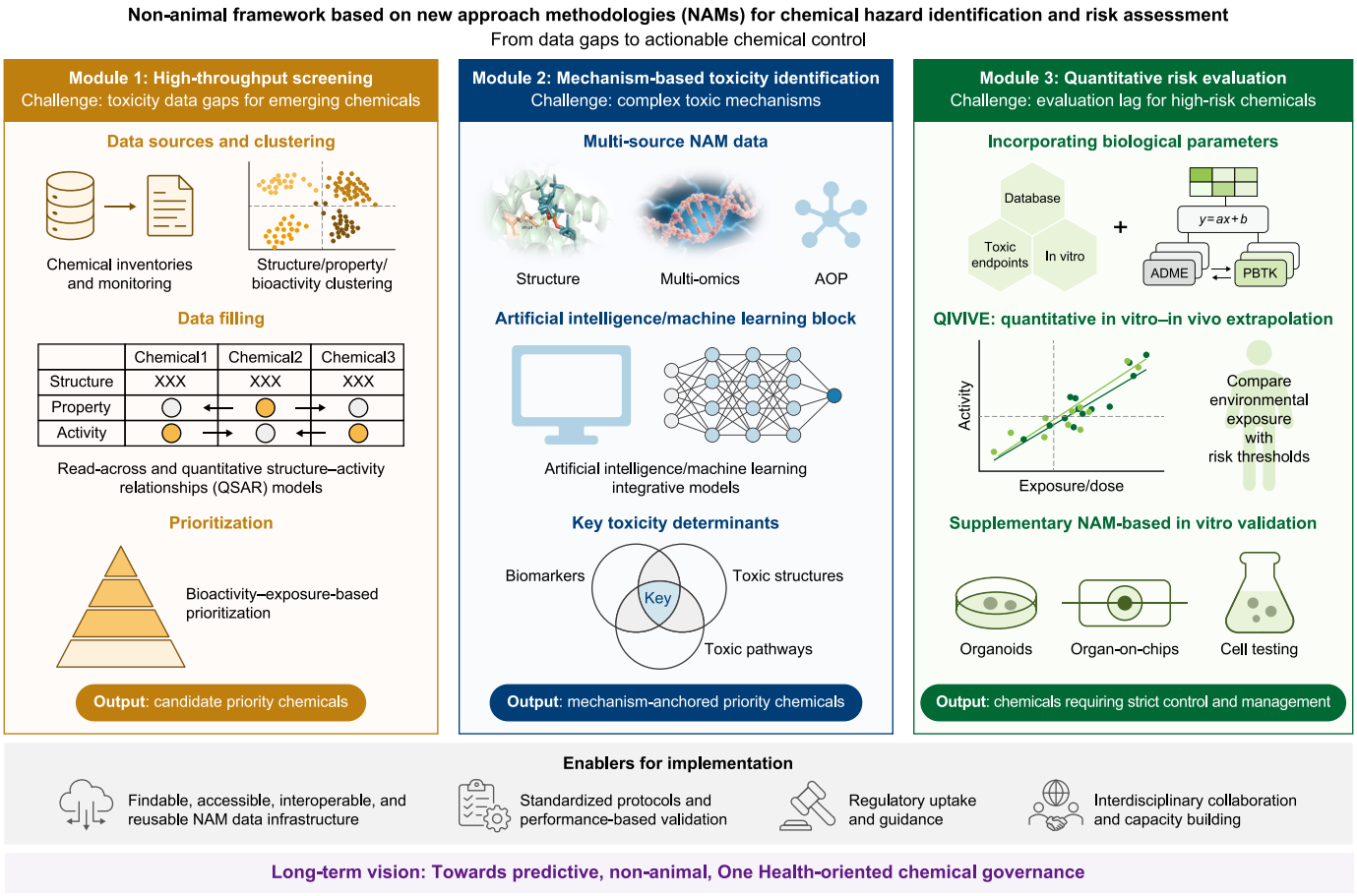

Fig. 1. Non-animal framework based on new approach methodologies (NAMs) for chemical hazard identification and risk assessment. The framework comprises three modules: (1) high-throughput screening to address toxicity data gaps and select candidate priority chemicals; (2) artificial intelligence/machine learning–enabled, mechanism-based identification of key toxic structures, biomarkers and pathways from multi-source NAM data (for example, multi-omics and adverse outcome pathways [AOPs]); and (3) quantitative risk evaluation that integrates absorption, distribution, metabolism and excretion (ADME), physiologically based toxicokinetics (PBTK) and quantitative in vitro–in vivo extrapolation (QIVIVE), supported by in vitro and organoid validation, to derive health- and ecology-based thresholds for chemicals requiring strict control.

are determined based on their structures, properties, and toxicity endpoints from multiple databases. For chemicals with insufficient or missing toxicity information, toxicity endpoints can be estimated using the read-across method and quantitative structure-activity relationships (QSAR) models. These toxicity endpoints enable the systematic selection of priority toxic chemicals. For instance, the OECD-recommended QSAR Toolbox contains physicochemical properties and toxicological endpoints for over 14,000 chemicals, supporting embedded read-across and trend analysis to fill the chemical data gap. The module enables efficient monitoring of emerging chemicals, facilitating proactive updates to the global chemical regulatory list and priority pollutant list, helping to overcome the current “tip of the iceberg” limitations in recognizing new pollutants.

Secondly, artificial intelligence (AI) models—including machine learning and deep learning—can be applied to extract unstructured data on priority toxic chemicals identified in the previous step. These unstructured data include multi-omics information (genome, transcriptome, and proteome) and adverse outcome pathways. Furthermore, these models can capture complex structure–activity relationships and visualize multi-level interactive networks of pollutants, targets, genes, pathways, and phenotypes. The quality of model input data can be improved by evaluating the rationality of the experiments, the sample size, and the credibility, as well as performing dimensionality reduction to optimize the input features. Model limitations, such as data bias, overfitting, and poor interpretability, can be addressed through appropriate data cleaning, control of iteration numbers, incorporation of model constraints, and the addition of explanatory layers. For example, Jiang et al. [9] developed ToxACoL, a multi-condition acute toxicity prediction model that reliably predicts toxicity endpoints in data-scarce and cross-species scenarios. ToxACoL addresses challenges arising from overcoming diverse experimental conditions, imbalanced datasets, and limited target data. This module enables high-efficiency identification of key toxic structures, biomarkers, and toxic pathways of the priority toxic chemicals. This step conducts a more rigorous screening of priority toxic chemicals by integrating evidence on their toxicological mechanisms, thereby refining and further narrowing the list.

Finally, the risk assessments of identified priority toxic chemicals require incorporating cross-species or individual physiological variability parameters, physiological toxicokinetic (PBTK) models, and considerations of absorption, distribution, metabolism, and excretion. Then, a quantitative in vitro–to-in vivo extrapolation model is constructed to establish an in vivo exposure–dose–response relationship. This model translates in vitro effect concentrations into in vivo equivalent doses in animals or humans, thereby yielding sensitive ecological or health thresholds. Xie et al. [10] predicted the corresponding in vivo exposure threshold and no-effect concentration of chemicals by applying the PBTK model

of fish physiology combined with in vitro–in vivo extrapolation, providing insights for the ecological risk assessment of chemicals. Environmental exposure concentrations of pollutants are shared and obtained through Internet of Things technologies. The risk is quantified by comparing the environmental exposure concentration with sensitive thresholds, addressing the problem of delayed chemical risk assessment. When physiological or toxicological data gaps persist, high-throughput data can be generated through in vitro cell testing and microphysiological systems, including organoids, organ-on-a-chip devices, and three-dimensional cell culture models. These new technologies also contribute to correcting the deviations in toxicity prediction models and efficiently verifying certain key toxicity mechanisms of chemicals, especially in the regulatory assessment of chemicals. Overall, the non-animal framework represents a transition from retrospective to predictive assessment paradigms. It is expected to address key limitations of traditional chemical governance—including slow detection, delayed assessment, and limited control—thereby safeguarding public health, maintaining ecological security, and supporting sustainable development.

The application of the non-animal framework in toxicology remains challenging. In terms of model data, multimodal data often contain substantial noise, insufficient standardization, and a lack of sharing mechanism, resulting in reduced model training accuracy. If toxicity data are generated through sequential predictions across the three modules, the associated errors may accumulate and amplify. Implementing FAIR (findable, accessible, interoperable, and reusable)-compliant standardized data repositories across institutions enables evidence-based predictive toxicological modeling. Incorporating a non-animal framework into the regulatory framework requires rigorous scientific justification and evaluation. The shortage of interdisciplinary talents and high-cost pressure constitute impediments at the resource level. It is necessary to strengthen interdisciplinary collaboration, policy inclination, and financial support.

In conclusion, we propose a unified and scalable framework for chemical hazard assessment that extends beyond piecemeal applications of NAMs. The non-animal framework can quantify health risks of chemicals to both animals and humans, as well as identify and manage highly polluting chemicals. It will support environmental decision-making, optimize governance strategies, and minimize pollution and ecological health risks caused by chemicals to the greatest extent possible. By embodying the principles of One Health, the framework promotes the deep integration of toxicology, ecology, and public health, enabling a paradigm shift from single-species safety assessments to the optimization of the global health systems. Ultimately, it aims to provide more comprehensive and sustainable solutions to global challenges such as climate change, biodiversity loss, and environmental pollution. Reductions in pollutant emissions will be beneficial for protecting biodiversity and carbon sinks (such as soil and forests), reducing greenhouse gas emissions, and strengthening ecosystem resilience.

CRediT authorship contribution statement

Tuantuan Fan: Writing - Original Draft, Investigation. **Zhenfei Yan:** Methodology, Investigation. **Chenglian Feng:** Writing - Review & Editing, Conceptualization, Methodology. **Fengchang Wu:** Writing - Review & Editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Dr. Fengchang Wu, the Vice Editor-in-Chief of *Environmental Science and Ecotechnology*, was not involved in the editorial review or the decision to publish this article.

Acknowledgment

This work was funded by the National Natural Science Foundation of China (Grant Nos. 42277274 and 42394154).

References

[1] United Nations Environment Programme, Making peace with nature: a scientific blueprint to tackle the climate, Biodivers. Pollut. Emerg. (2021). https://www.unep.org/resources/making-peace-nature. (Accessed 16 October 2025).
[2] R. Fuller, P.J. Landrigan, K. Balakrishnan, G. Bathan, S. Bose-O'Reilly, M. Brauer, J. Caravanos, T. Chiles, A. Cohen, L. Corra, M. Cropper, G. Ferraro, J. Hanna, D. Hanrahan, H. Hu, D. Hunter, G. Janata, R. Kupka, B. Lanphear, M. Lichtveld, K. Martin, A. Mustapha, E. Sanchez-Triana, K. Sandilya, L. Schaefli, J. Shaw, J. Seddon, W. Suk, M.M. Téllez-Rojo, C. Yan, Pollution and health: a progress update, Lancet Planet. Health 6 (2022) e535–e547, https://doi.org/10.1016/S2542-5196(22)00090-0.
[3] Z. Yan, X. Jin, C. Feng, K.M.Y. Leung, X. Zhang, Q. Lin, F. Wu, Beyond the single-contaminant paradigm: advancing mixture toxicity and cumulative risk assessment in environmental toxicology, Environ. Sci. Technol. 59 (2025) 10711–10714, https://doi.org/10.1021/acs.est.5c05712.
[4] L. Yang, Y. Wang, Y. Sun, R. Yu, Y. Chu, Y. Yao, C. Liu, N. Li, L. Chen, J. Liu, Z. Zhao, H. Zeng, CO_2-Responsive smart wood scaffold for natural organic matter removal without secondary pollution, Adv. Mater. 37 (2025) 2505008, https://doi.org/10.1002/adma.202505008.
[5] C.P. Nelson, P. Brown, S. Fitzpatrick, K.A. Ford, P.C. Howard, T. MacGill, E.E.C. Margerrison, J. O'Shaughnessy, T.A. Patterson, R. Raghuwanshi, R. Rouse, S. Stromgren, K.E. Sung, L.G. Valerio, J.L. Ward, N.N. Bumpus, Advancing alternative methods to reduce animal testing, Science 386 (2024) 724–726, https://doi.org/10.1126/science.adg6228.
[6] CompTox Chemicals Dashboard, United States Environmental Protection Agency, 2024. https://www.epa.gov/comptox-tools/comptox-chemicals-dashboard.
[7] K.-J. Jang, M.A. Otieno, J. Ronxhi, H.-K. Lim, L. Ewart, K.R. Kodella, D.B. Petropolis, G. Kulkarni, J.E. Rubins, D. Conegliano, J. Nawroth, D. Simic, W. Lam, M. Singer, E. Barale, B. Singh, M. Sonee, A.J. Streeter, C. Manthey, B. Jones, A. Srivastava, L.C. Andersson, D. Williams, H. Park, R. Barrile, J. Sliz, A. Herland, S. Haney, K. Karalis, D.E. Ingber, G.A. Hamilton, Reproducing human and cross-species drug toxicities using a liver-chip, Sci. Transl. Med. 11 (2019) eaax5516, https://doi.org/10.1126/scitranslmed.aax5516.
[8] J. Strickland, E. Haugabrooks, D.G. Allen, L.B. Balottin, Y. Hirabayashi, N.C. Kleinstreuer, H. Kojima, C. Nishizawa, P. Prieto, D.E. Ratzlaff, J. Jeong, J. Lee, Y. Yang, P. Lin, K. Sullivan, W. Casey, International regulatory uses of acute systemic toxicity data and integration of new approach methodologies, Crit. Rev. Toxicol. 53 (7) (2023) 385–411, https://doi.org/10.1080/10408444.2023.2240852, 2023.
[9] J. Lu, L. Wu, R. Li, M. Wan, J. Yang, P. Zan, H. Bai, S. He, A. Bo, ToxACoL: an endpoint-aware and task-focused compound representation learning paradigm for acute toxicity assessment, Nat. Commun. 16 (2025) 5992. https://www.nature.com/articles/s41467-025-60989-7.
[10] R. Xie, Y. Xu, M. Ma, Z. Wang, Fish physiologically based toxicokinetic modeling approach for in Vitro–In vivo and cross-species extrapolation of endocrine-disrupting chemicals in risk assessment, Environ. Sci. Technol. 58 (2024) 3677–3689, https://doi.org/10.1021/acs.est.3c08314.

Tuantuan Fan[a,b], Zhenfei Yan[a], Chenglian Feng[a,b,*], Fengchang Wu[a]
[a] *State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing, 100012, China*

[b] *College of Water Sciences, Beijing Normal University, Beijing, 100875, China*

[*] Corresponding author. Chinese Research Academy of Environmental Sciences, Anwai Dayangfang No. 8, Chaoyang District, Beijing, 100012, China.
E-mail address: fengcl@craes.org.cn (C. Feng).

18 August 2025